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Effect of food and micronutrient supplementation during pregnancy on
subsequent development of infants in Bangladesh: A randomized trial

Fahmida Tofail

Submitted for the Degree of Doctor of Philosophy

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Centre for International Child Health

Institute of Child Health

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Abstract

Background: The prevalence of low birth weight (LBW) is high in developing countries and is estimated to be 30% (UNICEF, 2001) in Bangladesh. Maternal under nutrition is an important cause of LBW is also highly prevalent in Bangladesh 50 % (March of Dimes, 2002). Public health programs of food-supplementation during pregnancy have been mounted to address the issue and it is important to determine the most effective way of providing the food. In addition it has been suggested that supplementation with multiple micronutrients may be more beneficial than supplementing with iron and folic acid alone, which is the present practise. Most studies of pregnancy supplementation have focused on the effect on birth outcomes whereas there is extremely little data on the effects on the offspring's' development.

A large randomized-trial of the effect of 2 types of nutritional supplementation (food and micronutrients) in pregnant women on birth-outcomes was conducted in the Matlab field-site of ICDDR,B: Centre for Health and Population Research, Bangladesh. We took the opportunity to evaluate the effect of the supplements on children's development.

Aims: We aimed to determine the effect of giving pregnant women early (around 10 week of gestation) versus late (around 17 week of gestation) food-supplementation and multi-micronutrients or 30mg iron + 400 µg folate or 60mg iron + 400 µg folate on their infants' development.

Methods: A sub-sample of all singletons (n=2853) born between May 2002 and December 2003 in the main trial was selected to have developmental assessments at 7 months of age. The children were assessed using 2 problem-solving tests (cover and support), the Bayley motor-scale (PDI) and Wolke's behaviour ratings assessing approach, activity, emotionality, co-operation and vocalization during the test procedure. The children were also assessed for the age of attainment of motor milestones.

Intervention: 2 nutritional interventions were given-

Food supplementation: Women were randomly assigned to begin the food supplementation program (a) immediately after diagnosis of pregnancy (early care) or (b) at the time of their choosing (usual care).

Micronutrient supplementation: Within each food group, women were randomly assigned to receive a pill that contained (a) 30 mg iron and 400 µg folate or (b) 60 mg iron and 400 µg folate (usual care) or (c) 30 mg iron, 400 µg folate and 13 additional micronutrients (UNICEF/WHO/UNU, 1999 formulation of 15-micronutrients).

Results: There is no overall benefit of prenatal supplementation with early food or multiple-micronutrients compared with late food or iron and folate supplementation on any of the tests of children's development when assessed at 7 months of age.

However infants of thin mothers (body mass index $<18.5 \text{ kg/m}^2$) showed a small but statistically significant benefit from both early food and multiple-micronutrients supplements whereas the children of better nourished mothers did not. Early food supplementation benefited children of malnourished mothers in the problem solving tests, support (BMI x early food $p<0.03$) and cover (BMI x early food $p<0.05$) and behaviour. The children were less fussy (BMI x early food $p<0.04$), more cooperative with the test situation (BMI x early food $p<0.04$) and vocalised more often (BMI x early food $p<0.04$) than children of similar mothers given late food supplementation. Small but significant benefits on motor development (BMI x micronutrients $p=0.05$) and activity (BMI x micronutrients $p<0.05$) were also observed among the infants of malnourished mothers who received multiple-micronutrient supplements. Mothers' BMI had an independent effect on children's development.

Conclusions: Early food and multiple micronutrient supplementation benefited development in children of undernourished mothers but not children of better nourished mothers. The findings support current practices in Bangladesh of targeting thin mothers and suggest that early food supplementation may be more beneficial than later supplementation.

The findings also suggest that multiple micronutrients may be more beneficial to the child than iron and folate. However, the effect sizes were very small and their clinical and public health importance are not clear and can only be determined with longer follow-up. As there was no placebo group, the benefit of giving food supplementation throughout pregnancy could not be assessed. The relationship between mothers BMI and children's development emphasises the importance of maternal nutrition.

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I remain grateful to these organizations.

Dedication

To my Parents:

Father Brig. General Tofail Ahmed

and

Mother Jahanara Tofail

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Abbreviations Used

ACC/SCN	Administrative Committee on Co-ordination/Sub-Committee on nutrition
AGA	Appropriate for Gestational Age
API	Appropriate Ponderal Index
BINP	Bangladesh Integrated Nutrition Programme
BMI	Body Mass Index (weight in kg / height in m ²)
BNAS	Bazelon Neonatal Behavior Assessment Scale
BSID-II	Bayley Scale of Infant Development
BW	Birth Weight
CI	Confidence Interval
CNC	Community Nutrition Centre
CNP	Community Nutrition Promoters
DfID	Department for International Development
DPC	Dorsolateral Prefrontal Cortex
DQ	Developmental Quotient
ECD	Early Childhood Development
FFQ	food Frequency Questionnaire
FT-II	Fagan Test of Infant Intelligence
HIV	Human Immuno-deficiency Virus
HOME	Home Observation for Measurement of Environment
ICDDR,B	International Centre for Diarrhoeal Disease Research, Bangladesh
INCAP	Institute of Nutrition of Central America and Panama
IQ	Intelligence Quotient
IUGR	Intrauterine Growth Retardation
LBW	Low Birth Weight

LMP	Last menstrual period
LPI	Low Ponderal index
MDI	Mental Development Index
MRC	Medical Research Council
NBW	Normal Birth Weight
NNP	National Nutrition Programme
OFC	Occiputo-Frontal Circumference
OR	Odds Ratio
PDI	Psychomotor Development Index
RCT	Randomised Controlled Trial
RDA	Recommended Daily Allowances
RR	Relative Risk
RE	Retinol Equivalent
SD	Standard Deviation
SE	Standard Error
SES	Socio Economic Status
SFD	Small-for-Dates
SGA	Small for Gestational Age
SMA	Supplementary Motor Area
UNICEF	United Nations Children's Fund
UNU	United Nations University
WAZ	Weight for Age Z score
WHO	World Health Organisation
UNICEF	United Nation's International Children Emergency Fund
WHO	World Health Organisation
WHZ	Weight for Height Z score

Chapter 1: Literature Review

1.1. Introduction

This thesis concerns an extension of an ongoing longitudinal, randomised controlled trial known as the “Maternal and Infant Nutritional Intervention at Matlab (MINIMat)” study. The study looks at the effect of three combined public health nutritional interventions with 5,000 pregnant women living in a poor rural area in Bangladesh. Two of the interventions were given during pregnancy and compared the effect of supplementation with 1) early and late food supplementation and 2) supplementation with one of three different micronutrient mixes on birth outcomes. Following delivery the mothers also participated in a trial of breast feeding counselling. This thesis concerns a subsample of the women's offspring and examines the effect of the interventions during pregnancy (not the breast feeding counselling) on their early development and is known as the *child development component*. In particular, I examined the children's problem solving ability, motor development and behaviour at 7 months of age. The effect of the breast-feeding counselling is not examined in this thesis. In addition I examined the time of acquisition of certain fine and gross motor milestones from 3 months to 7 months of age to describe the pattern in Bangladesh.

In this chapter I will discuss

- ☉ Early child development
- ☉ The prevalence and aetiology of maternal under nutrition and low birth weight (LBW)
- ☉ The development of small for gestational age (SGA) infants
- ☉ Nutritional supplementation during pregnancy and child development
- ☉ The situation in Bangladesh and the description of the study area
- ☉ Justification for the present study

1.2. Early child development

1.2.1. What is child development?

Development is defined by Holt as ‘...a process of unfolding, expanding, becoming fuller, more complex and more complete’ (Holt, 1991).

Development of human beings differs in some respects from development of other mammals. According to Sheridan (Sheridan, 1978), the four outstanding biological achievements of Homo Sapiens indicating development are –

- (i) Attainment of the “upright posture” which allows movement (locomotion), changing postures and allowing the hands to be free
- (ii) “Visual competence” and “ability to use flexible digits” – means ability to do specialized fine motor activities (holding pen to write, sewing, putting thread into needle-hole etc) with flexible fingers (digits) guided by vision
- (iii) “Spoken language”
- (iv) “Evolution of complex societies”

Child development is defined in a simpler term by Myers as “a process of change in which the child learns to handle ever more complex levels of moving, thinking, feeling, and relating to others” (Myers, 1995). The process of development is distinctive and unique in children, and distinguishes children from adults (Pollak, 1993). However it is not straightforward and not easy to determine. Gesell described an infant as “...a growing action system” (Gesell & Amatruda, 1941).

Child development is a multidimensional and integral process. Its important dimensions include physical, motor, language, social and emotional development. No single dimension or aspect of development can alone adequately describe a child. For example, a child’s ability to distinguish a round from a square shape describes something about his visuo-spatial skills, but hardly tells anything about the many other behavioural aspects of which he is capable. All dimensions of development are interrelated and one dimension affects the development of the other dimensions.

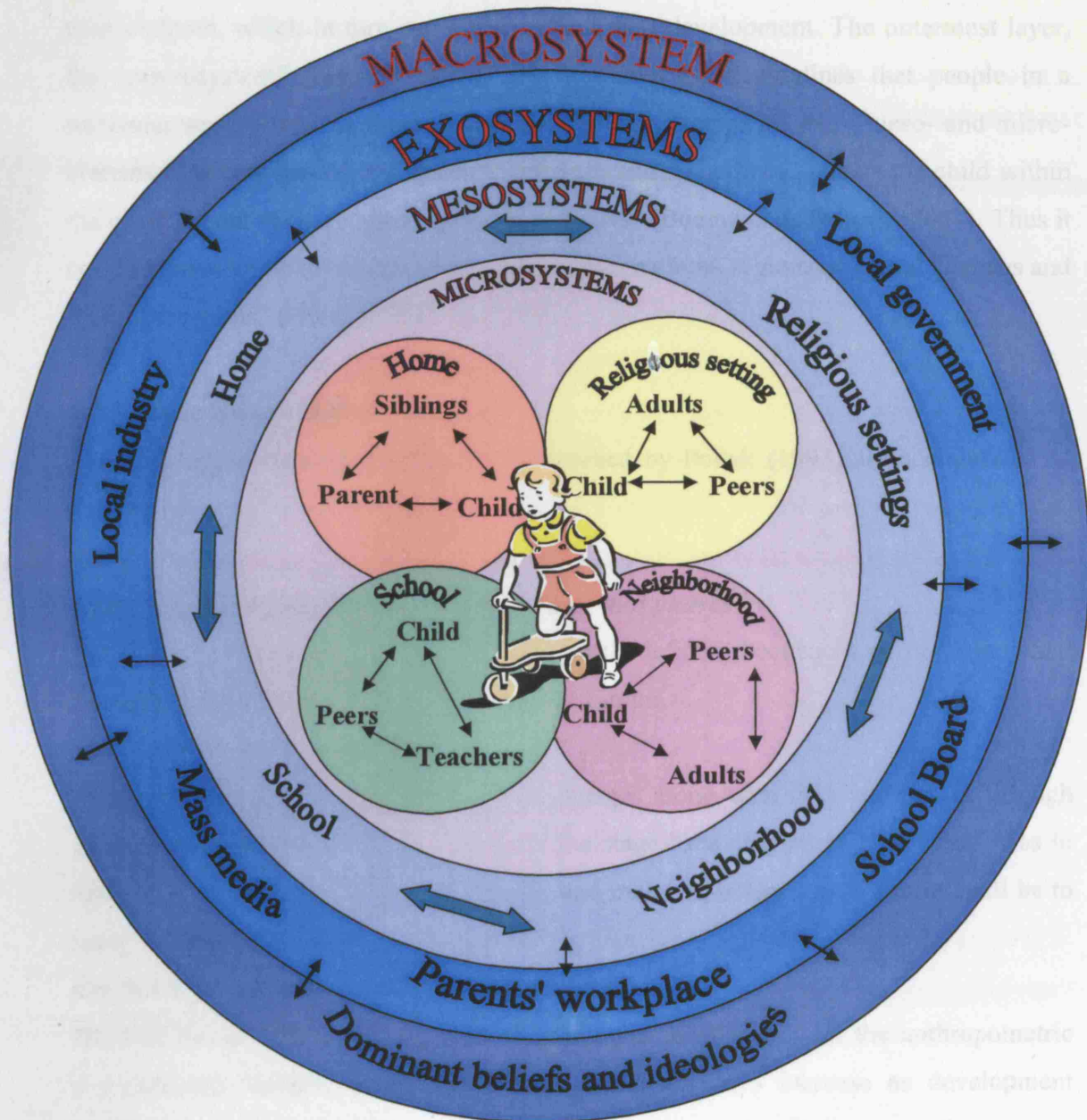
1.2.2. Developmental context

The “context of development” actually refers to many interrelated things. Urie Bronfenbrenner described these many developmental contexts in a conceptual framework of concentric rings, where each ring influences those inside it. This model helps to identify the factors which influence and shape development (Bronfenbrenner, 1979). Bronfenbrenner’s “Ecological Systems Theory” was recently elaborated and renamed as “Bioecological System Theory” (Boemmel & Briscoe, 2001). In the conceptual model (Figure 1.1.) the child lies at the centre of the concentric rings with his or her own biological make-up, containing various systems within him or herself. Surrounding the child is the first ring “microsystem”, the immediate environment, containing the settings of people, and the physical objects with which the child has direct contact. This “microsystem has the most immediate effect on the child” (Boemmel & Briscoe, 2001). It includes parents, peers, non parental adults (teachers, caretakers), family members or anyone who has an intimate relationship with the child for a considerable amount of time. It also includes the home environment, toys (availability, variety etc), playground, classroom etc.

The microsystem is immediately surrounded by a layer known as the “mesosystem” that concerns the intermediate level of influences and their interactions. According to Berk (2000) this layer concerns “...connection between children’s immediate setting and surroundings...It encompasses connections between microsystems such as home, school, neighbourhood and child-care centre, that foster children’s development”.

All these settings are embedded in a broader “social and economic context” that affects the child indirectly. This layer is known as “exosystem” (Boemmel & Briscoe, 2001).

Figure 1.1. The bioecological context of child developmental



The ecological approach sees children in the context of all various settings they inhabit on a daily basis (Microsystems). These settings are related to one another in a variety of ways (mesosystems), which are in turn linked to settings and social institutions where the children are not present but which have an important influence on their development (exosystems). All of these systems are organized in terms of culture's dominant beliefs and ideologies (the macrosystems) (Bronfenbrenner, 1979; Moen, Elder and Lüscher, 1995) (Source: Michael Cole, 3rd edition, p 20)

This layer concerns social settings like parents' workplace, local government or the community. For example, parents with unstable jobs may not spend quality time with their children, which in turn can hugely affect their development. The outermost layer, the "macrosystem", consists of all beliefs, values and guidelines that people in a particular society tend to share. The macrosystem envelops all exo-, meso- and micro-systems. The contents of this system "influence and sometimes support the child within the environment such as cultures, norms and laws" (Boemmel & Briscoe, 2001). Thus it covers "the most removed influences such as international regions or global changes and their interactions" (Huitt, 1999).

1.2.3. Basic laws of child development

The nine "basic laws of development" described by Pollak (1993) are summarized as follows:

(i) Development goes through "defined stages and phases"

All normal children pass through specific, well defined, sequences of developmental changes (milestones), which are constant and similar.

(ii) Development "infers change"

It suggests that each developmental skill changes along with time and passes through past, present and future. For example, for the stage "standing alone" the "past" was to learn how to put body weight on both legs and maintain balance and "future" will be to learn "walking".

(iii) Development "includes a physical increase in size"

Physical increase in size along with development is obvious. All the anthropometric measurements (height, weight, chest size, head size etc) increase as development progresses until adult life. However this increment may not be always proportionate.

For example, head circumference increases at a slower rate than the other body parts and becomes $1/8^{\text{th}}$ of adult body size, and was $1/4^{\text{th}}$ at birth.

(iv) Association of physical growth with "increase in function and ability"

This indicates that along with increment in physical size, functional ability of a particular organ also improves. For example, increase in muscle-mass and strengthening

of bony structures enables the infant to improve their random limb-movement to more organized creeping movement and finally to more synchronized walking.

(v) Development involves “maturation”

Along with growth, maturation of an organism is also essential to development. Myelination is one such essential maturational process that continues throughout life and plays an important role in acquisition of many motor developmental skills (Gesell & Amatruda, 1965).

(vi) “Development and maturation takes time”

As mentioned earlier, development strictly progresses in a timely order in normal populations. We cannot expect equal expertise from two children of two different ages so the age of testing is important when measuring intelligence e.g. comparing behaviour of a two year and a three year-old child with a form board shows that a two year old child can easily place a round shape in a round hole but not when the board is rotated whereas a three year old can do it. However that does not mean that the three year child is more intelligent than the two year old child.

(vii) Certain components of development (motor development) maintains a direction (cephalocaudal)

Motor development in humans is cephalo-caudal (from head to foot) and proximo-distal (from lips-tongue, then eye muscle then neck-shoulder-arms etc). Thus a child first acquires neck control then lifts its chest followed by the sitting and upright positions.

(viii) Development progresses from “general to the specific”

This transformation occurs with time after the child passes through developmental differentiation to specialization. For example, a child grasps the cube with its whole hand (general) initially at four months of age then progresses to more specific thumb-fore finger grasp by the end of the first year.

(ix) Development has a “compound interest effect”

Pollak (1993) describes this law of development as “Human development consists of complicated and intricate processes, each new phase building upon the sum of previous ones and the phases and stages of this development are orderly and inevitable”. Piaget showed that early reflex “schema” provide a skeleton for mental structure. This skeleton gradually entraps experiences and transforms to get a shape through adaptations. This

enables the child to acquire a particular skill. For example, experience gained from the primitive “sucking reflex” gradually helps a child to modify it and drink from a cup.

1.2.4. Pre and post natal development

Development is a continuous process that starts in the womb of the mother and continues throughout life. Development at an early stage of life is very important because what occurs in early life may affect development in later life. As development of nervous system continues throughout intrauterine life e.g. neurogenesis during early pregnancy and brain growth spurts during late pregnancy, it has been postulated that any insult at this period is harmful and may have long lasting effect on cognition and behaviour.

Here I will briefly discuss first “prenatal” and then “postnatal” development focusing on brain development and cognition.

(i) Prenatal development

The prenatal period can be divided into three periods - the germinal period, the embryonic period and the foetal period. Table 1.1. shows the developmental events of embryo at different time points.

Prenatal periods

- ***Germinal period***

In the 2 week period following conception, repeated cell division occurs to form a hollow ball of identical, proliferating cells called a blastocyst which burrows itself into the uterine bed. Then within a few days, the blastocyst differentiates into an “embryonic disc”. It is a three layered structure and each layer is further programmed to form major organ systems. The inner layer (endoderm) differentiates to form the internal organs - heart, lung etc, the middle layer (mesoderm) mainly forms skeletal and muscular structures and the outer layer forms nervous system and skin.

- *Embryonic period*

The embryonic period extends from week 2 through to week 8. All major organs and parts of the body develop by maintaining a strict timetable, in a very predictable order e.g. during week 4, the heart starts to beat and the neural tube closes, in week 5 arm and leg buds form, in week 7 facial structures fuse and in week 8 major development of the organs is complete.

- *Foetal period*

The embryonic period is followed by the fetal period that extends from week 9 until birth. During this period body parts grow rapidly and refinement occurs. The developing organism greatly increases in size and starts moving, sleeping, waking and “breathing”.

Table 1.1. Developmental events of the embryo at different time points

Time after conception	Major events
12-13 days	Implantation complete
14 days	Mature placenta begins to develop
3 weeks (15-20 days)	Development of three-layered disc Neural tube begins to form Disc becomes attached to wall by short, thick umbilical cord Placenta develops rapidly
4 weeks (21-28 days)	Eyes begin to form. Heart starts beating Crown-rump length 5 mm; growth about 1 mm per day Neural tube closes Vascular system Placenta maternal-infant circulation begins to function
5 weeks	Arm and leg buds form
7 weeks	Facial structure fuse
8 weeks	Crown-rump length 3 cm Major development of organs completed Most external features recognizable at birth are present

Prenatal brain development

At the beginning of the third week after conception, the nervous system starts to develop. A portion of ectoderm (neural plate) begins to fold on its own to form a hollow tube like structure known as a “neural tube” and continues to differentiate in all directions. Longitudinally it presents 3 initial subdivisions which differentiate to form a forebrain, a midbrain and a spinal cord. Fusion of the neural tube occurs in a cephalocaudal direction and completes at around the 25th day after conception. The forebrain and midbrain further differentiate to form numbers of bulges and convolutions whereas the spinal cord differentiates to form a series of segments. Around the fifth week, the different parts of the human brain become distinguishable – bulges and convolutions of fore- and mid-brain represent the cortex (telencephalon), thalamus and hypothalamus (diencephalons), midbrain (mesencephalon), pons and cerebellum (metencephalon) and medulla (myelencephalon). In the brain and spinal cord the ventral part deals with sensory pathways and sensory cortex, whereas the dorsal aspect deals with the motor pathways and the motor cortex (Johnson, 2005).

The wall of the recently closed neural tube consists of a type of lining of epithelial cells known as “neuroepithelial cells”. Soon after the closure of the “neural tube”, along the radial dimension the tube develops a zone called the “proliferative zone” which deals with cell proliferation, migration and differentiation of “neuroepithelial cells” to a particular type of cell. This zone produces precursor cells – neuroblast and glioblast that differentiates to neurones (nerve cells) and glial cells (support and supply cells) respectively. Each neurone contains cell body and cytoplasmic extensions- an axon and dendrites. Dendrites branch repeatedly but axon extends as a single process. After formation, newly formed neurones migrate from the “proliferative zone” to the particular area of brain where they can perform their specific task and form a mature brain. Like neuroblasts, glioblasts also migrate to different parts of brain and differentiate to form different types of supporting cells like-protoplasmic astrocytes, fibrillar astrocytes, oligodendroglia etc (Johnson, 2005; Sadler, 2000). These cells provide support, act as insulators and under pathological condition phagocytose. Astrocytes are found around the blood vessels wall and thought to regulate ion-transport

and act as blood-brain barrier (Chatterjee, 2004). During elevation and folding of the neural plate, a group of cells of ectodermal origin appear along each edge of neural folds, known as the “neural crest”. These neural crest cells form “Schwann cells” that wrap themselves around long extensions (axons) of peripheral nerves – a process known as myelination. Synapses are the specialized area of contact between two or more neurones and the cleft at the junction is known as synaptic cleft (20-30nm wide), where transmission of nerve impulse takes place. Larger synaptic vesicles usually contain catecholamines (adrenaline, nor-adrenaline and dopamine) and smaller vesicles contain acetylcholine.

The cortex in all mammals is made up of 6 layers with some basic characteristics. The first layer is composed of long white fibres connecting different areas of the brain. Layers 2/3 also have connecting fibres mainly projecting forward from pyramidal cells of adjacent cortex. Layer 4 is composed of the termination of most of the input fibres. It also contains star shaped cells that are connected to the fibres. Layers 5/6 are composed of major output fibres and giant pyramidal cells. These basic structures of the cortex are more or less the same all over the brain except some regional variation exists based on their function. For example, the input layer (layer 3) is thick in the visual cortex and one of the output layers (layer 5) is thick in the motor cortex (Johnson, 2005).

(ii) Postnatal development

In this section I will briefly discuss the brain, cognitive and motor development of infants. Assessments of postnatal cognitive and motor developments we further discussed in the methodology section in chapter 3. Other aspects of postnatal development that involve personal-social development, adaptive (non verbal) development, communication and language development, learning, hearing and vision will not be discussed here.

Postnatal brain development

Postnatal brain growth is slow and prolonged in humans which allows interaction with the environment to modify the “brain’s circuitry” (Johnson, 2005). In contrast to new

cell formation, massive brain growth occur during this period due to an increase in neuronal connections (synapses), nerve fibres (dendrites) and fibre bundles. In addition covering all these fibres is a fatty sheath (myelination) which contributes to an increasing brain volume. There is a general consensus that by two years of age the brain structure simulates the overall appearance of the adult brain and by three years of age all main fibre tracts are visible (Matsuzawa et al, 2001; Paus et al, 2001). At birth most neurones have migrated to their appropriate location though some neurogenesis in some brain structures like the hippocampus continues (Eriksson et al, 1998). The visible “sulci and gyri” of brain at birth also remains immature in their inter- and intra-regional connectivities (Johnson, 2005).

(iii) Some important events occur during neuronal development

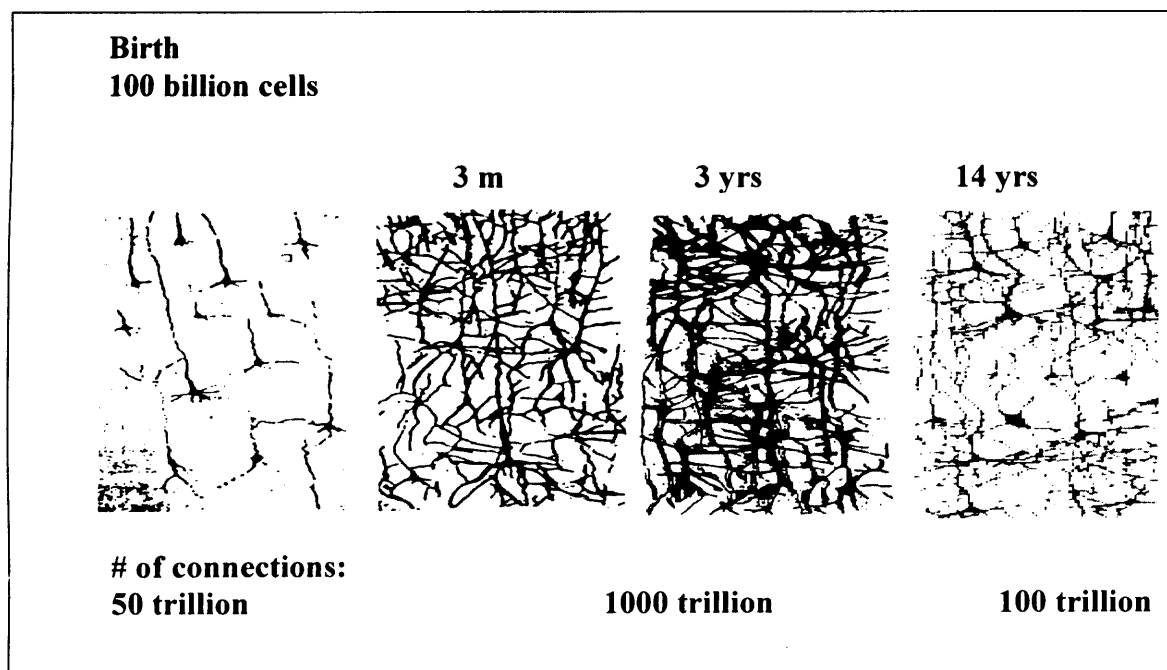
There are several important development processes in the brain including neuronal migration, dendritic proliferation, neurotransmitter development and myelination.

Neuronal migration: Neuronal migration almost entirely takes place during prenatal period in humans. Neurones in the cortex arise from the zone adjacent to the ventricle (ventricular zone) of the developing brain. After cell division they migrate outward from the ventricular zone by moving along the fibres of special glial cells known as radial glia, which stretches from the ventricular zone to the surface of the developing cortex. In the cortex, the young cells remain in the superficial layer and older cells remain in the deeper layer (Gazzaniga et al, 2002). Around seven months of gestation the majority of neurones have migrated to the appropriate brain regions. According to Sadler (2000), “once neuroblasts form, they lose their ability to divide”. However, some addition of neurones has been reported in the hippocampus and else where postnatally.

Dendritic proliferation: There is an increase in size and complexity of dendritic trees known as dendritic proliferation which begins around the time of birth to make them more specialized and specific and increase synaptic contacts (synaptogenesis) between cells. Rapid bursts of synaptogenesis start in different cortical areas at different ages and are later followed by dendritic and synaptic pruning. Dendritic pruning also occurs at

different stages in different brain regions. Rapid synaptogenesis occurs in the visual and primary auditory cortex at 3 to 4 months with maximum density reached between 4 to 12 months and is reduced to adult level around 2-4 years. In contrast, synaptogenesis and dendritic proliferation is slow in the prefrontal cortex and reach its peak around first year and adult level between 10-20 years. It is suggested that the initial increase in dendritic proliferation and synapses play an important role in the plasticity (ability of nervous system to change) of young brain (Johnson, 2005). Figure 1.2. shows the rapid increase in connections after birth. The functional consequences of selective dendritic loss (pruning) are not clear but it has been suggested that it is regulated genetically and by sensory input. Functional specialization results in separation of particular areas of the nervous system from neighbouring regions known as 'percellation', where dendritic or synaptic loss probably plays a role (Johnson, 2005). Neuronal plasticity is very important for many coordinated learned movements (e.g. ordinary activities, athletics, movements during music etc).

Figure 1.2. Cellular structure showing increase in neuronal connection after birth *Source: Conel (1967)*



Neurotransmitters: These substances are glycoproteins or lipoproteins, they are synthesized by the endoplasmic reticulum and packed as electron dense vesicular granules in the Golgi bodies of specialized neurones. Formation begins around 28 days of gestation after the neurones have migrated. After formation they are transported to the terminal end of axon for secretion. They can be intrinsic, produced inside the cortex or extrinsic, produced outside the cortex. Intrinsic transmitters can be excitatory or inhibitory. Glutamate, aspartate, catecholamine, acetylcholine and serotonin are some excitatory transmitters and gamma-amino-butyric acid (GABA), glycine and taurine are some inhibitory transmitters (Datta, 1995; Gazzaniga et al, 2002). Extrinsic neurotransmitters include acetylcholine, formed mainly in the basal forebrain, norepinephrine, formed in the locus ceruleus, serotonin formed in the brainstem raphe nuclei and dopamine, formed in the substantia nigra. Factors like temperature, mechanical pressure, blood supply, chemicals (carbon-di-oxide and narcotics like ether, chloroform etc), hydrogen-ion concentration, and oxygen lack affect nerve conductivity and excitability. Changes in certain cations (Ca^+ , K^+ and Mg^+) also affect neurotransmitter function. Most intrinsic and extrinsic neurotransmitters are present in the cortex at birth and show a pattern of increasing postnatally then declining. In the rat brain glutamate receptors increase between 10-15 postnatal days to reach a peak of 10 times the adult level and then falls by the 25th postnatal day. Similarly the density of GABA receptors, in the mammalian brain increases rapidly in the perinatal period, doubles in the first few weeks and then declines. It has been reported that the levels of GABA can be influenced by the extent of sensory experience. In several mammals density of noradrenergic fibres in the cortex is very high at birth compared to density seen in adults. In rhesus monkey, the serotonin fibres reach adult levels by the 6th post natal week, continue to rise there after then fall (Johnson, 2005). In contrast dopaminergic fibres show the adult pattern of projections in frontal and singulate cortex throughout the post natal period.

Myelination: Myelin is a complex mixture of lipid and protein. The lipid component is mainly cholesterol, lecithin, glycolipid, galactocerebroside and phospholipids. The myelin sheath is composed of many layers of modified cell membrane, derived from

Schwann cells in peripheral nerves and from oligodendrocytes in the brain. Myelinated fibres facilitate nerve conduction and act as insulators with high electrical resistance. Myelination is important to increase the ability of information transmission by the neurones and it is hypothesized that myelination in some areas is linked with behavioural development. However recent research showed that under-myelinated connections are also capable of transmitting signals (Johnson, 2005). Myelination of nerve fibres begins at different times in different parts of brain. It is most prominent during the period of rapid growth and begins approximately two months after differentiation of neurones. The first part to myelinate is the peripheral nervous system and motor roots myelinate before sensory roots, followed by primary somesthetic (touch), visual and auditory cortices (Nelson, 2000). In the first 3 postnatal months the secondary association areas around the primary sensory and motor cortices begin to myelinate. The final areas to myelinate involve higher cortical functions and are mainly in the frontal cortex. These areas begin around the 4th postnatal month and continue to as late as mid adolescence.

In summary, prenatal brain development is mainly concerned with the formation of neurones (brain cells) and their migration to appropriate brain regions. This task is almost entirely completed around the seventh month of the prenatal period in humans.

Cognitive Development

Vasta et al (2004) described “cognition” as “all the higher order mental processes by which humans attempt to understand and adapt to their world – processes such as thinking, reasoning, learning and problem solving”. For survival, and to fulfil our desires, we need to learn how to manipulate our environment with full control and cognitive development helps learning (Goswami, 1998).

Piaget’s view on cognition - Jean Piaget, through his competent observations of his own children, developed an original theory of cognitive development during infancy and childhood (Piaget & Inhelder, 1956). He claimed that infants lacked innate physical knowledge, and knowledge was acquired through observing the effect of their actions.

Thus children are active participants in that learning process throughout infancy. He described cognitive development in qualitative nature by stage theories as follows:

- **Sensory motor stage (birth-2 years)** - At this stage infants develop more control over their sensory perceptions and motor behaviour. Here cognition is based on action and key achievements are developing such as “object-permanence” (Goswami, 1998). They learn how to elaborate their early reflexes (sucking, rooting, grasp etc) and random movements to voluntary actions. They pass through 6 sequential defined sub stages during this time period as described below.

Stage 1 (birth-1.5 months): This sub-stage consists mainly of an involuntary reflexive prepackaged set of actions (schemas) like sucking, rooting, stepping.

Stage 2 (1.5-4 months): This is a sub-stage named by Piaget as “primary circular reaction” where the infant finds the repetition of random actions pleasurable.

Stage 3 (4-8 months): Also known as “secondary circular reaction”. Infants develop a concept of the environment. They become aware of the relation of their own actions to environment that produces interesting changes.

Stage 4 (8-12 months): Infants can coordinate means and ends; they can combine schema to achieve a desired effect and try to solve simple problems.

Stage 5 (12-18 months): Also known as “Tertiary circular reaction”. The child deliberately experiments in order to see the consequences of an action.

Stage 6 (18-24 months): The child invents new means of problem solving through mental combinations using symbols.

- **Pre-operational thought (2-6 years)** - At this stage children can think about images and symbols and can elaborate on them, but cannot make general statements.
- **Operational thought (6-12 years)** - “The key achievement of this stage is the understanding of transitivity - the ability to organize entities into an ordinal sequence via the use of logical inferences based on the relation between them.” (Goswami, 1998).

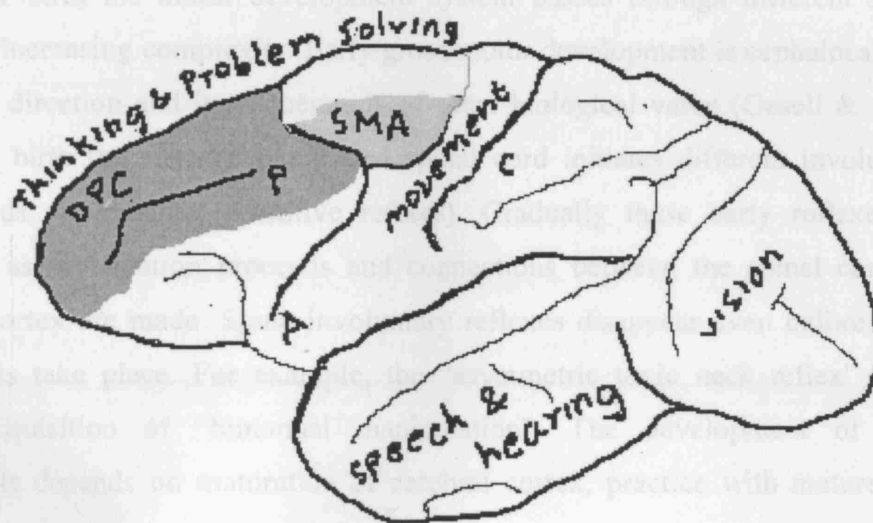
- Formal operational thought (12+ years) - At this stage children develop the ability to generate a hypothesis and explain it logically with scientific thoughts. Thus the key achievement at this stage is “...ability to reason by analogy ” (Goswami, 1998).

Modern views of cognition - However views of modern researchers contrast to Piaget’s theory of cognition. Goswami (1998) has argued that if infants lack object permanence they would have to live in a constant ‘snapshot world’ of the here and now and would therefore have difficulties with several basic mathematical concepts, such as ordinal relation and measuring. There is now substantial evidence that young infants do possess a core of knowledge about the physical world and are capable of reasoning (Baillargeon, 1993; Willatts 1997). These new “core knowledge” and “active representation” hypotheses highlight infants’ innate capacity of thinking and interpreting the outcome of a physical event. For example, they have innate knowledge about solidity of an object. They know that it should not pass through another solid object, needs support to remain in position, and that its movement follows a continuous path etc. Evidence revealed that sophisticated cognitive abilities such as visual and auditory perception develop very early in life. Slater et al (1983) reported ability of neonates to distinguish a circle from a cross. Some cognitive skills like memory and learning even start to function in the womb around the 3rd trimester (DeCasper & Fifer, 1980; Goswami, 1998).

There still remain questions as to why, even after having such a sophisticated understanding about object concept and spatial relationship, do young infants face great difficulty in solving problems and demonstrating their knowledge of object concepts and spatial relationships in their behaviour? It has been suggested that neural maturity, acquisition of motor skills and ability to inhibit reflexive reactions to contact (grasp reflex), all play a role in such cognitive performance (Diamond, 1991). Experiments have shown that around the period of 5-8 months of age infants develop the ability to inhibit their “reflexive reactions to contact” due to maturation of their “supplementary motor area (SMA)” in the cerebral cortex. Between 8-12 months of age they learn to

inhibit their “predominant response tendency (i.e. tendency of reaching directly for a visible goal without thinking for a more efficient way)”, and this behaviour depends on maturation of “dorsolateral prefrontal cortex (DPC)”.

Figure 1.3. Lateral view of brain showing supplementary motor area and dorsolateral prefrontal cortex



SMA= supplementary motor area is the light shaded area just behind the arcuate sulcus, which extends further to the midline than can be shown in the diagram
DPC= dorsolateral prefrontal cortex, is the dark shaded area just in front of the arcuate sulcus, it centres around the principal sulcus and extends upto the frontal pole
C= central sulcus, all cortex in front of central sulcus is part of frontal cortex
P= principal sulcus. This is the heart of dorsolateral prefrontal cortex
A= arcuate sulcus, this is the principal boundary between supplementary motor area and dorsolateral prefrontal cortex
(Source: Diamond, 1991, p.69)

Around the same time, development of “relational ability” occurs with the ability to relate (bimanual co-ordination) two different simultaneous movements (e.g. to get a toy on a cloth, pulling a cloth with one hand and picking a toy with the other hand). This is thought to be due to the development of inter hemispheric connections in the corpus callosum between the two SMAs on each side of the brain (Fig 1.3.). At the age of 8-12 months considerable advances occur in the ability to relate information over time and space. The skills of memory and sustained attention are required to link information separated in space or time. For this developmental skill, maturation of the DPC is

required for remembering a sequence of actions and SMA for executing a sequence of actions (Diamond, 1991).

Motor Development

Motor development concerns how children use their body such as posture, prehension, balance and movement. It is usually divided into gross and fine motor development. Soon after birth the motor development system passes through different stages and phases of increasing complexity. Early gross motor development is cephalocaudal (head to tail) in direction and its sequence is of great biological value (Gesell & Amatruda, 1947). At birth the already myelinated spinal cord initiates different involuntary and spontaneous movements (primitive reflexes). Gradually these early reflexes start to disappear as myelination proceeds and connections between the spinal cord and the cerebral cortex are made. Some involuntary reflexes disappear even before controlled movements take place. For example, the “asymmetric tonic neck reflex” disappears before acquisition of “bimanual manipulation”. The development of voluntary movements depends on maturation of cerebral cortex, practice with mature postures, acquiring increased control on muscle, limb movements and balance (Pollak, 1993).

Gross motor activities include mainly large muscle movements e.g. sitting, crawling, walking etc. Early head stabilization enables the infant to explore its surroundings with vision and hearing. Gradually other gross motor skills develop in different postures (prone, supine or upright). In normal healthy children motor development in different postures occurs simultaneously and they can easily change from one posture to another. Developing the new motor-skill is suggested to occur due to progression from a previous motor activity in the same posture, not necessarily progression of a similar skill exhibited in another posture (Holt, 1991). For example, walking develops as a progression of walking with support and standing without support in the upright posture, not a progression of the early stepping reflex. These skills are further supported by involvement of particular muscles and kinesthetic stimuli arising from the different actions (Holt, 1991). The same muscle can be used differently for different motor activities. For example, at around 9 months of age, short extensor hip muscles are

relaxed in the sitting posture with a brief contraction to maintain the posture. In contrast these muscles contract alternatively to extend the thigh at the hip joint when the child attempts to crawl. A fairly similar pattern of early motor development (motor milestone) is observed in most of the normal children, however they might skip one or two milestones, e.g. hand-knee crawl. Table 1.2. shows the advantages of acquiring motor skills.

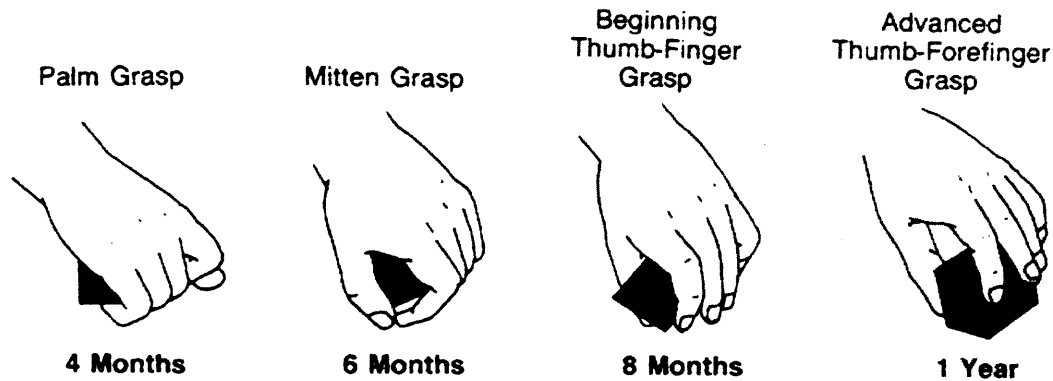
Table 1.2. Advantages of mobility in infancy

Physical	(a) Enhance physique-circulatory effects, increase muscle strength, etc (b) Practice in development of skills- sensory, motor co-ordination, balance, etc.
Intellectual	(a) Exploration- spatial awareness, promotion of audiovisual development, increased learning opportunities, etc (b) Communication- more effective, wider scope, etc.
Emotional	(a) Internal stimulation and enjoyment (b) Interpersonal relationship is enhanced and controlled

(Source: Holt, 1991, p.76)

Fine motor development includes the ability to use the hands, fingers and thumbs with increasing dexterity, precision with skills like pincer grip and picking small objects etc. These movements are complex and the skills involve strength, speed, eye-hand co-ordination and perception (Pollak, 1993). Fine motor skill develops later after development of some gross motor skills, particularly after head control. Posture is important for fine motor activities which are facilitated by the sitting posture and hampered by the prone posture or crawling. This is the motor skill that differentiates man from most animals and gradually progresses from general to specific (Fig 1.4.) along with maturation of the motor area in the cerebral cortex.

Figure 1.4. Developmental progresses from “general to the specific”



[Development of grasping during 1st year]

1.2.5. Factors influencing development

(i) Factors influencing prenatal development

Prenatal development unfolds partly in a “hereditary context” which includes inherited genes from each parent. It also partly unfolds in an “environmental context”, that includes the biochemical environment which surrounds the organism and maternal factors as follows.

Biochemical environmental factors

Some biochemical agents which can alter the environment around the developing organism and affect its development by preventing or modifying normal cell division and differentiation are known as “teratogens”. Usually this type of damage is selective (Pollak, 1993) and “timing” of exposure determines the extent of damage as well as the system that will be damaged. However, some systems such as the central nervous system grow and differentiate rapidly throughout gestation and are thus exposed to damage at any point of gestation. It has been reported that the first 2 to 8 weeks of post-conception is the most critical period of the embryonic stage and is known as the “foetal sensitive period” (Schaffer, 1985). This period is vulnerable to the developmental damage because rapid growth and formation of major body parts occurs during this time.

So teratogens are potentially more harmful at this stage than during the later part of the prenatal period when refinement of existing structure occurs. The classic example of Thalidomide clearly shows the importance of the timing of teratogenic insults. Mothers who took this drug after three weeks of conception had babies without ears, after 4 weeks had babies without legs or with deformed legs and after 8 weeks with no malformations.

The other examples of teratogens include drugs like aspirin (Kozier et al, 2002), some antinauseant drugs (Miklovich & van den Berg, 1976), alcohol (Sokol et al, 1980), hormones like diethylstilbestrol (Heinonen et al, 1977), tobacco (Butler & Goldstein, 1973; Rush and Callahan, 1989; Law et al, 2003), heroin and methadone (Bohlin et al, 1985; Bunikowski et al, 1998; Marcus et al, 1982), environmental toxicogens like arsenic or lead exposure (Hopenhayn et al, 2003; Ahmad et al, 2001) etc.

Maternal factors

Both physical and mental factors of mothers might influence the development of the growing foetus as follows.

- *Intrauterine infections:* Some infections during pregnancy such as HIV, rubella, toxoplasmosis etc can cause developmental malformation of the newborn.
- *Psychological factors:* Anxiety, mental illness, depression and emotional stress may affect the foetus. Anxiety might cause an increase in the metabolic expenditure, hormonal imbalance (catecholamine, cortisol etc) and loss of appetite. All these factors can ultimately lower gestational weight gain and cause preterm labour or delivery of low birth weight infants (Kramer, 1987). Emotional stress also causes behavioural changes in the offspring (O'Connor et al, 2003).

A recent review showed a positive association of antenatal maternal stress and anxiety with subsequent behavioural problems in neonates; sleeping, feeding and activity problems in infants; poor attention, hyperactivity and emotional problem in pre-school children; and lower intelligence in adolescents even after

controlling for social factors (Van den Bergh et al, 2005).

- *Maternal age:* The age of mothers is associated with genetic disorders. Older mothers over 40 years of age have less successful pregnancies and an increased incidence of babies with congenital anomalies (Wyatt et al, 2005).
- *Maternal protein energy malnutrition:* Poor maternal nutritional status including low gestational weight gain due to inadequate nutrient-intake and short stature as an indicator of childhood nutrition, are all considered as major determinants of low birth weight, particularly in developing countries (ACC/SCN, 2000a). An earlier study compared 25 newborns of severely malnourished mothers with 23 infants of well nourished mothers (Bhatia et al, 1979). The malnourished mothers had weight less than 25th percentile of weight for height with haemoglobin and serum albumin level less than 80 g/l and 25g/l respectively. The infants of malnourished mothers demonstrated gross intra-uterine growth retardation with significantly lower muscle tone and excitability compared to infants of well-nourished mothers. Low birth weight (LBW) infants are likely to have slightly slower cognitive and motor development, learning deficits and behavioural problems (Grantham McGregor et al, 1999). In human subjects, analysis of brain tissue of LBW infants who died soon after birth, also showed that significant reduction in brain weight, brain lipids (particularly myelin lipids) and cellularity compared to normal birth weight infants (died during postnatal period). The cerebellum was the brain area in human subjects that was affected most by malnutrition (Chase et al, 1972). Its weight reduced by 37% and cellularity 35% compared to a 21% and 19% reduction of other parts of the brain in LBW infants respectively. Myelin lipids, cerebroside and sulfatide were also reduced significantly ($p < 0.01$) more in the cerebellum than other parts of the brain. Further details of the effect of maternal nutrition (role of both prenatal micro and macronutrients) on subsequent growth and development and long-term consequences of LBW infants are discussed in the following section of this chapter.

- *Maternal Micronutrient deficiencies*

The effects of multiple-micronutrients on brain development are not well researched. However, on reviewing the function of the individual micronutrients during pregnancy on the development of the nervous system of the offspring, I found that several of them have been reported to have an effect on the brain development. The studies come mainly from animal models and involve iron, folate, thiamine (vitamin B1), pyridoxine (B6), copper and zinc.

Iron: It has been reported that in animal models the developing brain is particularly sensitive to severe iron deficiency and irreversible behavioural changes occur (Felt & Lozoff, 1996). Brain iron content, myelination, dopamine metabolism and brain enzymes are all affected. The brain has a high iron content which is unequally distributed throughout the brain (Moos, 2002). IDA in 10 day old rats results in 30-40% lower brain iron which is not remedied with iron treatment (Youdim, 2001). Most studies are in the post natal period but Kwik-Uribe and colleagues (2000) showed that chronic marginal iron deficiency during the second and third trimester and lactation in rats lead to pup brains that were deficient in iron, showed changes in dopamine metabolism and myelin composition and some of the effects were not reversible with iron given postnatally. Iron deficiency also affects enzyme systems. For example the following enzymes are reduced: ribonucleotide reductase which regulates brain growth, delta-9 desaturase which regulates myelination, tyrosine hydroxylase which regulates dopamine D2 receptor synthesis and cytochromes which regulate energy production (Tanaka et al, 1995; Connor, 1994; Yu et al, 1986). Cytochrome C oxidase (CytOx) is required for the last stage of oxidative phosphorylation reaction and de Ungria and colleagues (2000) showed that it is significantly reduced in brain areas involved with higher cognition in iron deficient neonatal rats. The areas affected included hippocampus, dentate gyrus, cingulate cortex, dorsal thalamic nucleus and the piriform cortex that specifically deals with memory processing.

Folate: It is well established that periconceptional folate deficiency is associated with neural tube defects and increased risk of preterm delivery, intrauterine growth restrictions and low birthweight (Fernandez-Ballart & Murphy, 2001; Scholl & Johnson, 2000).

Thiamine: Thiamine deficiency is not uncommon during pregnancy in developing countries. No trials of this vitamin in human subjects could be located. Heinze and Weber (1990) reported that mothers who had significantly lower concentrations of thiamine in red blood cells and plasma during the 28th and 29th week of pregnancy delivered infants with severe IUGR. Thiamine deficiency in animal studies has also been reported to cause severe IUGR, reduced placental weight and reduced liver weight (Roecklein et al, 1985; Levin et al, 1985). More importantly thiamine deficiency in animals during pregnancy has been reported to cause impaired foetal brain development. The impairment results from deficiency of thiamine-dependant enzymes required for cerebral energy metabolism and myelin synthesis (Fournier & Butterworth, 1990). Deficiency in pregnant women of thiamine also causes anorexia, lassitude, muscle weakness ataxia and tachycardia (Institute of Medicine, 1990).

Pyridoxine (B6): Pyridoxine (B6) acts as a co-enzyme in decarboxylation and transamination of amino acids. Adequate functioning of the nervous system depends on pyridoxine in animal models. Vitamin B6 deficiency during gestation and lactation alters the function of the neurotransmitter system and plays an important role in learning and memory (Guilarte, 1993; Kirksey & Wasynczuk, 1993). Animal studies have also shown that B6 deficiency during pregnancy and lactation can cause structural impairment in the hippocampus (Krishna & Ramakrishna, 2004) and epileptiform seizures and movement disorders due to biochemical and morphological abnormalities (decreased dendritic arborization and reduced numbers of myelinated axons and synapses) in brain (Gerster, 1996). Severely vitamin B-6 deficient human infants showed similar behavioural abnormalities. Neonates with marginal deficiency were also found to have a

lower birth weight and to display less mature reactive and adaptive behaviour in the Brazleton Neonatal Assessment Scale than well-fed infants (Gerster, 1996).

Zinc: Zinc is an essential trace element, a constituent of many metallo-enzymes involved in a wide range of metabolic processes. Its deficiency is associated with impaired growth and development, decreased appetite and taste sensation, pregnancy complications and reduced immune response. Zinc is present in the brain, bound to protein and is important for its structure and function (Sandstead, 1986). Zinc is present in high concentrations in synaptic vesicles of the special “zinc containing neurones” of the forebrain (Hesse, 1979; Frederickson et al, 1990, 2000; Howell et al, 1984). Zinc is a key modulator of neuronal excitability by facilitating the release of neurotransmitters like GABA (Ben-Ari & Cherubini, 1991) and glutamate. It also seems to have a role in neurogenesis, neuronal migration and synaptogenesis and deficiency hampers neurotransmission and subsequent neuro-physiological development (Colvin et al, 2000; Frederickson et al, 2000). However it is not clear whether supplementation with zinc during pregnancy benefits the offspring’s growth and development (Section 1.5.2)

Iodine: It is well established that iodine, an important component of thyroid hormone, is essential for growth and neurological development (Section 1.5.2). Severe deficiency during intrauterine life causes cretinism with reduced brain weight with a reduced number of cells. Morphological changes are apparent in the cerebellum, and cerebral hemispheres, with reduced migration of cells in some areas. There is also a reduction of myelination in the cerebral hemispheres. It is well documented that severe iodine deficiency in pregnancy has deleterious effects on cognitive and motor development. Moderate iodine deficiency causes lesser cognitive and motor deficiencies but brain changes are not well established. Thus providing mothers with sufficient iodine during childbearing age and pregnancy will prevent iodine deficiency disorders in children.

Copper: Severe copper deficiency in pregnant ewes resulted in neonatal ataxia (sway back) in their lambs (Institute of Medicine, 1990). This disorder results from the decreased activity of a copper-dependent enzyme (cytochrome oxidase) in the central nervous system. In rodents born to copper deficient dams showed missing cerebella, focal necrosis of cerebral cortex and lesion in corpus striatum (Carlton & Kelly, 1969). Menkes disease, a foetal neurological degenerative disorder caused due to missing enzyme P type ATPase that facilitates transfer of copper through cells (Danks et al, 1972). In recent animal study Penland and Prohaska (2004) showed permanent impairment of motor function in litters of rat that persists after long-term recovery from prenatal copper deficiency.

Vitamin E Vitamin E is a potent anti-oxidant and its deficiency has been reported to cause anaemia, neuromuscular abnormalities and reproductive failure (Institute of Medicine 1990). In humans its deficiency has been demonstrated in premature infants, mainly causing haemolytic anaemia (Oski & Barness, 1967) but it did not reduce preterm delivery in supplementation trials (Institute of Medicine 1990). There is evidence that vitamin E supplementation in newborns is protective in reducing intraventricular haemorrhage (Chiswick et al, 1983) and microcephaly in rats (Thanaka et al, 1986). However I could not find any report showing an association of maternal vitamin E supplementation in pregnancy with reduced health problems in infants. But the foetus accumulates vitamin E when it accumulates fat during the last 8-10 weeks of gestation (Institute of Medicine, 1990). Its deficiency has also been reported to cause neurological abnormalities in patients with prolonged and marked fat malabsorption (Kelleher et al, 1987; Muller, 1986; Sokol et al, 1985).

Other micronutrients Some micronutrients like niacin and vitamin B12 have also been reported to have an effect on the nervous system; however their role in brain development is not clear. A few studies in children reported associations of vitamin B12 deficiency with delayed language, motor and cognitive development and slower reaction time in neuropsychological tests (Black, 2003; Schneede et

al, 1994; Louwman et al, 2000). In elderly patients vitamin B-12 deficiency has been associated with neurological involvements like dementia, facial palsy and subacute combined degeneration (Nogales-Gaete et al, 2004; Rousso et al, 2005; Osimani et al, 2005). The nervous symptoms caused by niacin deficiency in adults include depression, disorientation, insomnia and delirium (Institute of Medicine, 1990).

(ii) Factors influencing perinatal development

Among the perinatal factors, any type of complicated birth events like obstructed delivery, instrumental delivery and perinatal anoxia can cause brain injury with adverse developmental sequelae. Birth asphyxia is an important cause of later developmental problems (Brown et al, 1974).

(iii) Factors influencing postnatal and early development

Pollak (1993) divided the influences of postnatal factors affecting child development into two components - the human and the physical. The “human component” influences a number of children and covers all the factors that directly or indirectly are in contact with a child as described (earlier) by Bronfenbrenner’s recently elaborated “Bioecological System Theory” (Boemmel & Briscoe, 2001). Physical factors like illnesses and disabilities also affect a number of children. Wachs (1999) described three different ways how experiences during early life influence development in later life:

Sensitising: In this case early exposure to a risk factor makes the child sensitive to further risk factors in later life compared to previously unexposed children. For example, stunted children, who suffered from poor nutrition in early childhood, were found to be more sensitive to hunger at school age compared to adequately nourished children (Simeon & Grantham-McGregor 1989).

Blunting: In this case early exposure to risk factors reduces the child’s ability to benefit from future developmental opportunities. A study showed that stunted children, adopted before 2 years of age, achieved a higher intelligence in later life compared to those who were adopted after 2 year of age (Lien et al.1977).

Steeling: In this case early exposure to protective factors protects the child from later untoward experiences. For example, in the longitudinal study in Kauai it was shown that high risk children, who had a more competent caregiver in the 1st year of life, were protected from later adversity (Werner, 2000).

1.3. Prevalence and aetiology of maternal under nutrition and low birth weight (LBW)

1.3.1. Global situation of Under nutrition

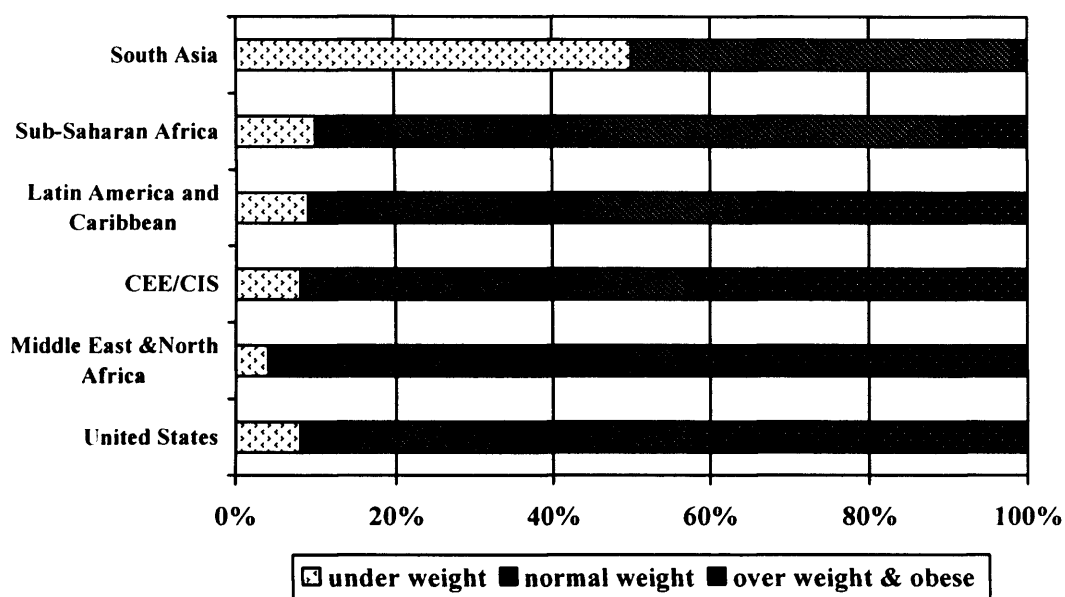
The magnitude of pregnancy and child-health related problems is alarming in many developing countries. Among the many problems, maternal nutritional status is regarded as one of the most important. Maternal weight during child bearing age is reported to be associated with pregnancy outcome (The March of Dimes, 2002) and possibly one of the main predictors of low birth weight (LBW), particularly intrauterine growth retardation (IUGR) (Kramer, 1987). Maternal underweight can cause an almost two-fold increase in the risk of foetal growth restriction (The March of Dimes, 2002).

The United Nations Administrative Committee on Coordination (UNACC), Sub-Committee on Nutrition (SCN) stated that “Because maternal undernutrition is a major determinant of LBW in developing countries, high rates of LBW should be interpreted not merely as an indicator of undernutrition, morbidity and mortality for the newborn, but as an urgent public health warning that women of child bearing age are undernourished as well” (UNACC, SCN, 2000). Body mass index (BMI) $<18.5 \text{ kg/m}^2$ is an indicator of thinness or undernutrition in adults (WHO, 1995) and as many as 40.5% women in South East Asia fall below this cut-off value (ACC/SCN,1992).

1.3.2. Maternal under nutrition

There is a high prevalence of underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$) in developing countries among women of child bearing age in contrast to a high prevalence of obesity ($\text{BMI} 25\text{--}29.9 \text{ kg/m}^2$) in developed countries (The March of Dimes, 2002).

Fig 1.5. Prevalence of underweight of women 20-49 years old by region countries



Under weight= $<18.5 \text{ kg/m}^2$; Normal weight= $18.5\text{-}24.9 \text{ kg/m}^2$; Overweight= $25\text{-}29.9 \text{ kg/m}^2$; Obese= $30\text{-}34.5 \text{ kg/m}^2$; Massively Obese= $>35 \text{ kg/m}^2$; CEE/CIS: Central Eastern Europe/Commonwealth of Independent States
 Source: Martorell et al (2000) and special analysis 2001 In: The March of Dimes, 2002

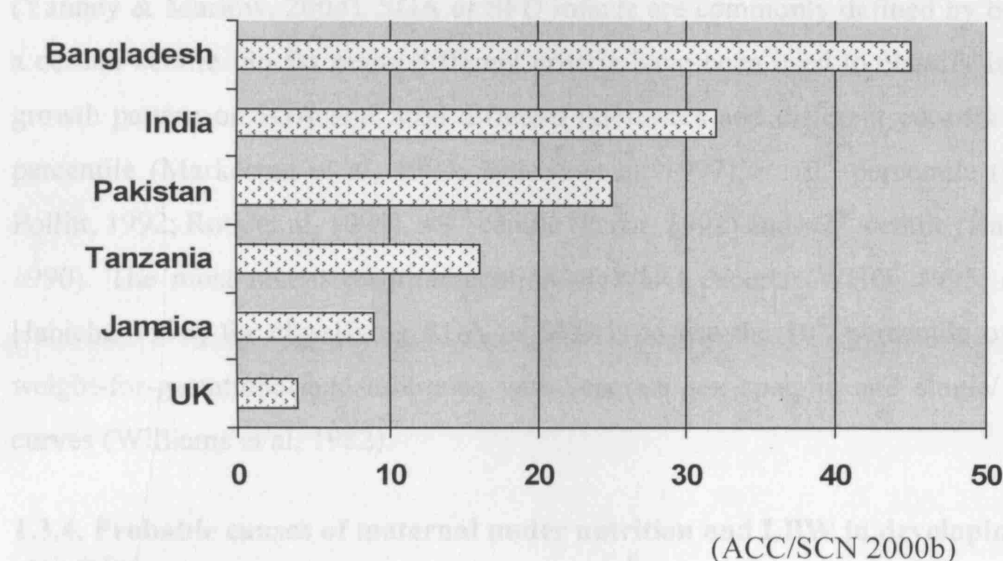
Among the South Asian countries (Figure 1.5.), more than 50% of women ages 20-49 years are underweight and in Bangladesh around 10% are severely underweight ($\text{BMI} < 16 \text{ kg/m}^2$). A country like Uzbekistan is in transition with 10% of women being underweight and 15% overweight, whereas in the United States $>40\%$ of women are overweight or obese (The March of Dimes, 2002).

1.3.3. Low birth weight

Similarly low birth weight (LBW $<2500\text{g}$) is a major problem worldwide. Almost 25 million LBW infants are born each year, 95% of them in developing countries where they are more likely to be small due to intrauterine growth retardation (IUGR) and mothers' under-nutrition and infection than to prematurity (Villar & Belizan, 1982). Around 80% full term LBW infants are born mainly in south-central Asia with the highest rate (almost 50%) in Bangladesh (ACC/SCN, 2000a).

The rate of term-LBW in middle and western Africa is 15% and 11% respectively (ACC/SCN, 2000a). Figure 1.6. shows the prevalence of LBW in some other countries of the world. The incidence rate of LBW >15% and IUGR >20% is high enough to create major public health problems and thus requires necessary intervention (de Onis et al, 1998; ACC/SCN, 2000a).

Figure 1.6. Proportion of infants with LBW in developed and developing countries



Definition and classification of birth weight

LBW: The latest definition of LBW with birth weight <2500g is well accepted and has been used for many years.

Maturity at birth: The definition of prematurity is more or less consistent and based on duration of gestation. If a child is born before 37 weeks of gestation, s/he is called “premature” or “preterm”. Birth with gestational age 37 weeks or more is considered as “term-birth”. However some investigators have used 36 weeks or 38 weeks for the cut-off for term/ preterm. In many studies the identification of IUGR has been confused with small-for-gestational-age (SGA) or small-for-dates (SFD) (Yanney & Marlow, 2004). However the terms SGA and IUGR are not strictly synonymous though there remains some overlap (Altman & Hytten, 1989). “An SGA birth is not necessarily an IUGR and an IUGR birth is not necessarily an SGA birth” (Bakketeig, 1998).

IUGR: No clear definition for IUGR has yet been agreed upon. It indicates a condition where growth restriction has occurred in utero due to some growth limiting factors, thus the foetus fails to grow at a predicted rate to attain its growth potential. The condition is mostly diagnosed clinically though it is now possible to monitor growth with ultrasound (Bakketeig, 1998). However IUGR infants may not be LBW (Yanney & Marlow, 2004). *SGA*: In contrast, small-for-gestational-age (SGA) or small-for-date (SFD) is a “statistical description of birth weight of babies born at a particular gestational age” (Yanney & Marlow, 2004). SGA or SFD infants are commonly defined by being below a certain centile. So far many different criteria have been used to classify intra-uterine growth pattern or SGA and used different references and different cut-offs e.g. < 15th percentile (Markestad et al, 1997; Nelson et al, 1997), < 10th percentile (Gorman & Pollitt, 1992; Roth et al, 1999), < 3rd centile (Pryor, 1992) and < 2nd centile (Hawdon et al, 1990). The most recent recommendation of WHO experts (WHO, 1995, de Onis & Habicht, 1996) for classifying SGA or SFD is to use the 10th percentile of the birth-weight-for-gestational-age reference with separate sex specific and single/ twins risk curves (Williams et al, 1982).

1.3.4. Probable causes of maternal under nutrition and LBW in developing countries

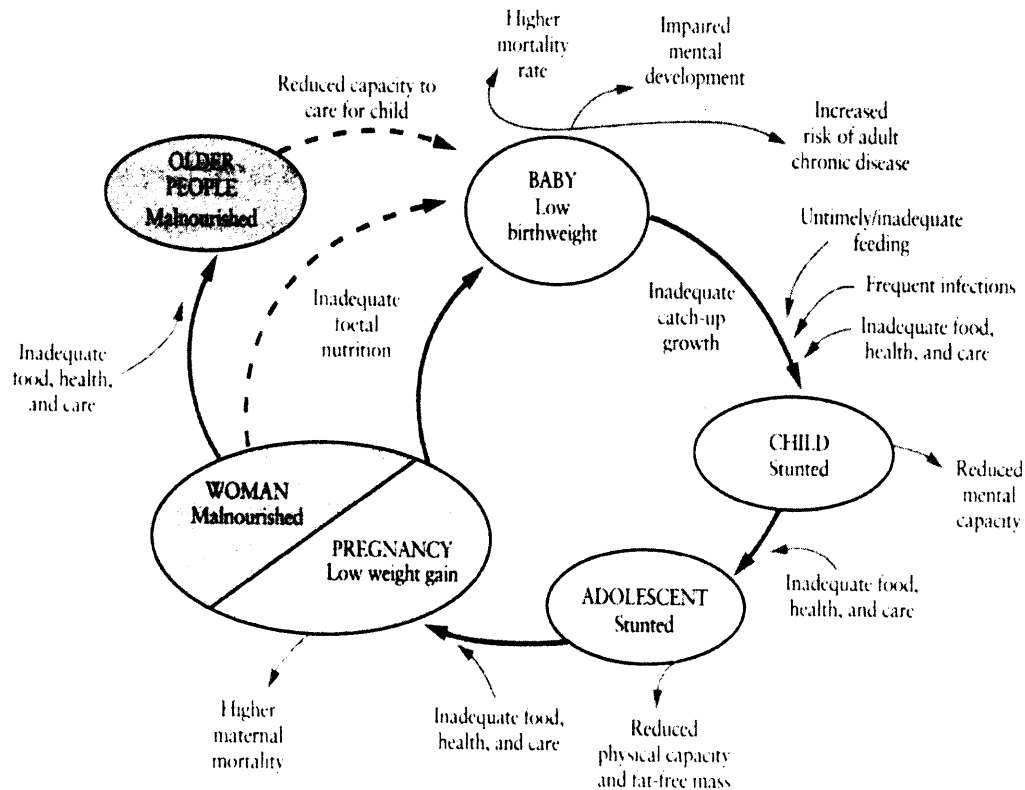
The problems of maternal under nutrition and LBW in developing countries are multifactorial in nature. The two major contributing factors that can cause maternal undernutrition are food intake and physical activities.

A child or adult fails to attain their growth potential if energy expenditure exceeds energy intake from consumed food (The March of Dimes, 2002). Deficiencies of different micronutrients as well as energy are also very common during pregnancy (Fernandez-Ballart & Murphy, 2001). Height and weight gain in female children and adolescents are considered as important precursors to attaining healthy adult BMI, ranging from 18.5-24.9 kg/m² (The March of Dimes, 2002). Early marriage and repeated frequent pregnancies in malnourished women make them reach pregnancy and lactation in a less than optimal state (ACC/SCN, 2000a).

If their increased demand for nutrients during pregnancy is not met, the growth and development of the foetus is likely to be affected (Figure 1.7.). Inadequate nutrient-supply during pregnancy is one of the important environmental factors that interferes with maternal-foetal exchange and results in adverse consequences in growth and development of the foetus (Rosso & Salas, 1994). Evidence from animal (ewes) studies shows that fasting for 5-7 days in late-gestation can cause reduction in both uterine and placental blood flow, (25% & 20% respectively) thus compromises the uptake of essential nutrients by the foetus (Morriss et al, 1980). Maternal fasting also showed alteration in both maternal and foetal metabolic changes along with foeto-maternal nutrient-exchanges which ultimately lead to severely compromised foetal growth (Mellor & Matheson, 1979; Lederman & Rosso, 1981).

It has been observed that prematurity accounts for most LBW babies in developed countries. In many of these cases the causes are unknown but bio-medical causes such as eclampsia, twin pregnancy etc can play a role. In contrast, prematurity accounts for a small percentage of LBW infants in developing countries. IUGR most commonly occurs in developing countries. Its causes are complex and multiple and may involve foetus, placenta and mother separately or in combination (ACC/SCN, 2000a). Evidence from epidemiological data show that pregnancy in chronically malnourished mothers result in shorter and lighter newborns with smaller head size, compared to well-nourished mothers (Arbuckle & Sherman, 1989; Thame et al, 1997).

Figure 1.7. Malnutrition cycle



Source: Commission on the Nutrition Challenges of the 21st Century (2000) Final report to ACC/SCN

Other probable causes of LBW in developing countries include exposure of mothers to micronutrient deficiency, stress during pregnancy, environmental hazards like lead pollution, arsenic contamination etc, toxic substances like tobacco etc (Kramer, 1987; Prada & Tsang, 1998; Fall et al, 2003). Similarly, influences of underlying adverse ‘immediate environment’ like poverty, status of women in the society, food insecurity, lack of care during pregnancy and poor access to health facilities also play an important role.

1.4. Low birth weight and child development

LBW is associated with increased perinatal complications as well as negative short and long-term health consequences (de Onis et al, 1998; Villar et al, 1984; Fitzhardinge & Steven, 1972; Starfield et al, 1982; Ashworth, 1998; Hay, 1996).

Although advancements in clinical management have resulted in better survival of the LBW infants, their developmental and neurological outcomes in later life remain to be understood (Wang et al, 1998; Hack et al, 2004, Fearon et al, 2004). In developed countries, the majority of researchers have reported adverse clinical outcomes and poor developmental consequences of very low birth weight (VLBW<1500 g) infants (Hack et al, 1991; Ross et al, 1985a; Saigal et al, 2000) as well as extremely LBW (ELBW <1000 g) infants with or without prematurity (Kilbride et al, 2004; Weindrich et al, 2003; Klein et al, 1972; Vohr et al, 2000; Saigal et al, 1991). A review of 80 studies, mostly in developed countries, also showed that LBW children (birth weight<2500 g) generally have poorer levels of development than normal birth weight (NBW) infants (Aylward et al, 1989). The development of term-LBW/SGA or IUGR infant is more complex and controversial. Evidence suggests that although this group of infants has a better chance of survival, subsequently they are at risk of behavioural problems (Fitzhardinge & Steven,, 1972), language difficulties (Walther & Ramaekers, 1982), attention-deficit and poor school performance, with or without affecting intelligence scores (Allen, 1984). As IUGR or SGA constitutes the majority of LBW from developing countries, I will focus my review on this group.

Search criteria: In this section I shall review the studies on infants born at term (gestational age 37 weeks or more) who were SGA or LBW (birth weight <2500 g) infants that compared their developmental outcome to normal birth weight (NBW; BW >2500 g) infants. An internet search was carried out for this purpose using PubMed. As many of the earlier studies have already been reviewed (Grantham-McGregor, 1998, Hack 1998), my search remained restricted to studies on term low-birth weight since 1990. I focused on all term-LBW/SGA studies with varying definition and selection criteria. I avoided studies that included preterm-LBW (gestational age <37 week and birth weight <2500 g) and very LBW (birth weight <1500 g) or extreme LBW (birth weight <1000 g) infants only. However, due to a limited availability of studies on developmental outcome in adulthood of SGA or term-LBW infants from developing countries, I included all available studies in the review.

First I shall describe the studies from developing countries in order of age of developmental assessments. Thereafter I shall describe the studies from developed countries in the same way.

1.4.1. Studies on the development of LBW/SGA infants from developing countries

Though there is evidence that the development of SGA babies is more vulnerable to poor environments where poverty is pervasive, surprisingly very few data are available on the developmental consequences of these infants from developing countries (Aylward et al, 1989; Grantham-McGregor, 1998; Grantham-McGregor et al, 1999), where poverty is more prevalent and the problem is more evident. I have summarized the details of these studies from year 1990 to date in Tables 1.3. to 1.4. I could locate only nine studies from developing countries that dealt with later development of full-term LBW or SGA infants.

Studies during infancy

Three studies from India, Brazil and Indonesia examined a developmental outcome during infancy (Table 1.3.). The Indian study (Padidela & Bhat, 2003) showed that term-LBW infants (gestational age >37 weeks, wt<2500g) had poorer neurobehavioural development on the Brazelton test at birth, but they had rapid improvement by the 14th postnatal day and scored even higher than appropriate for gestational age (AGA) babies in some developmental parameters. In Brazil (Grantham-McGregor et al, 1998) a larger, well controlled study on term-LBW infants showed significantly lower scores in mental developmental index (MDI) and psychomotor developmental index (PDI) compared to the normal birth weight (NBW) group at both 6 and 12 months of age. On the other hand the Indonesian longitudinal cohort study showed no difference on development of motor skills between NBW and LBW infants during their infancy (Kardjati et al, 1991). The details of this study could not be collected because only an abstract is available. In the abstract nothing is mentioned about the gestational age of these 561 infants, but their mean birth weight was 2850-2950 g and 9.5-12.2% of them were LBW infants.

Studies during childhood

Table 1.4. presents three studies from developing countries that assessed LBW infants between the ages of 2-15 years. A Guatemalan study (Villar et al, 1984, Gorman & Pollit, 1992) prospectively followed SGA infants (BW < 10th percentile) up to 5 years. They further grouped the SGA infants into adequate-ponderal-index (API ponderal index, PI=weight in g/length in cm³x100) group and low ponderal index LPI group. There was no difference in developmental scores between the 3 groups up to 15 months. At the age of 24 months the SGA-API group showed the poorest mental developmental performance though the difference was not significant, but at this age a subgroup of children (n=123), the SGA-API group, showed a significantly lower mental developmental score compared to NBW groups when adjustment of home-stimulation score and composite maternal index was made. By the age of 3 years, SGA-API infants scored significantly lower in 7 out of 8 subscales assessing “perceptual and problem solving performance”, “memory” and “verbal ability tests” than the SGA-LPI group and NBW group (Villar et al, 1984). In a further analysed verbal cognitive abilities and short term memory of the SGA-API group (n=41) were compared it to the NBW group (n=76) at the age 3, 4 and 5 years . At 36 months of age the SGA-API group had poorer scores in verbal cognitive abilities than the NBW babies but not thereafter. The interaction between SGA and early postnatal growth had a significant effect on short term memory at 36 and 48 months. SGA infants' were detrimentally affected by poor growth whereas NBW infants were not. At the age of 5 years no difference was observed between the groups. But by then the statistical power of the study was reduced significantly (Gorman & Pollitt, 1992).

One randomised control trial (RCT) in Jamaica (Walker et al, 2004) compared the development of 140 term-SGA infants with 94 NBW infants. SGA infants had two groups - one received, psychosocial intervention at home for 1st 8 weeks of life (n=70) and the other received none (control, n=70). All infants were assessed for developmental outcomes at the age of 7 months with problem solving tests (support and cover tests) and Wolke's behavior ratings and at the age of 15 and 24 months with the Griffiths Scales.

Control term-LBW infants of this study showed significantly lowered problem solving scores and behavior ratings compared to NBW infants at the age of 7 months. At the age of 15 and 24 months, they scored significantly lower scores in DQ and performance subscales compared to the NBW infants. They also scored lower in locomotor subscale at 15 months and hand and eye subscale at 24 months compared to NBW infants.

Another study in China (Jiang et al, 1991) compared early and late brainstem conduction time (BCT) in the brainstem auditory evoked responses (BAERs) between SGA and AGA infants. BCT is a reliable indicator of brain stem development and neurological function. They also compared early BCT (interpeak interval I-III) and late BCT (interpeak interval III-V) interval ratio on 178 appropriate-for-gestational-ages (AGA) and 24 small-for-gestational-age (SGA) children from birth to 6 years of age. The III-V/I-III interval ratio remained consistently smaller and significantly lower in SGA infants compared to AGA infants. The findings suggest that prenatal factors responsible for intrauterine growth retardation can cause suboptimal late or long-term development of the nervous system of SGA infants compared to AGA infants. However the number of SGA infants was very small.

Summary of term-LBW/SGA studies in developing countries

All six of the above-reviewed term LBW/SGA studies from developing countries assessed development in early childhood (Padidela et al, 2003; Grantham McGregor et al, 1998; Kardjati et al, 1991; Gorman & Pollitt, 1992; Walker et al, 2004; Jiang et al, 1991) and one of the early childhood studies followed the children into middle childhood (Gorman & Pollitt, 1992). In spite of wide variations in study design, population definition, assessment tools and control of confounders in the analyses, five out of six term LBW/SGA studies reported some sort of neurological, cognitive, behavioural or learning problems in LBW infants at sometime during childhood (Padidela et al, 2003, Grantham McGregor et al, 1998; Gorman & Pollitt, 1992; Walker et al, 2004; Jiang et al, 1991). These deficits were small but moderately consistent.

Only one study during infancy (Kardjati et al, 1991) failed to find any difference in motor development between LBW and NBW infants. However they compared a very small number of LBW infants with NBW infants as the prevalence of LBW was low (<12 %) in that population. Only an abstract was available for this study, so detail information could not be reviewed. Also the sizes of deficits appear to change over childhood and are not always present in the final year (Gorman & Pollitt, 1992). Unfortunately I could locate no studies on term LBW or SGA in developing countries that assessed developmental consequences over 6 years.

Usually studies in developing countries are confounded by a large number of environmental disadvantages. This makes it difficult to isolate the independent effect of LBW/SGA on development with certainty. Due to a large loss of sample (Gorman & Pollitt, 1992) over time, long term follow-up of these infants is difficult in developing countries. Earlier review of SGA infants (Grantham-McGregor, 1998) could not comment on developmental consequences of LBW infants in developing countries due to scarcity of information at that time. However since then a few more studies have been conducted on SGA infants from developing countries (Tables 1.3. to 1.4.) and their findings are moderately consistent. It is possible that these infants are more vulnerable to the adverse, impoverished and non-stimulated environment than NBW infants. The Jamaican study showed that the associated delay in development of term LBW infants can be minimised with psychosocial intervention (Walker et al, 2004). Thus there is a need for a long-term, well designed study on term LBW infants from developing countries to observe whether these early deficits resolve with time. Availability of information on long term consequences in these infants can further guide us with future interventions and policy implementations.

Table 1.3. Effect of SGA/term-LBW on child development and behaviour-studies from developing countries (age ≤1 years)

Study	Definition of groups	Sample/ Study Design	Assessment tool	Result	Comment
India Padidela & Bhat, 2003	SGA : Definition and how many of them were preterm not mentioned Mean BW = 2050±130gram Gestational age = 40.50±0.5 weeks Assessment On 3 rd , 7 th and 14 th day	Hospital Based case control study SGA = 38 infants NBW (BW: 2830±300 gram) =48 infants Controlled : Not mentioned	Brazelton Neuro-behavioural Assessment Scale (BNAS) on 3 rd , 7 th and 14 th day of life	BNAS On 3 rd day LBW infants scored significantly lower in habituation, range of state, autonomic stability and regulation of state clusters, compared to NBW infants. Only In orientation cluster, LBW scored significantly higher than NBW On 14 th day LBW infants scored significantly lower in habituation, motor organization, range of state, clusters than NBW. But they scored significantly higher in orientation, autonomic stability and regulation of state cluster than NBW	BW positively correlated neonatal behaviour Small number of cases with selection bias. Criteria for SGA not mention
North-east Brazil Grantham Mc-Gregor et al, 1998	Term-LBW: Birth Weight <2,500 gram Gestational Age : ≥ 37weeks Assessed =6 month & 12 months	Longitudinal cohort compared two groups LBW: n = 131 apparently healthy infants (BW: 1500-2499 kg) from maternity clinic NBW (control): n= 131 individually matched infants (BW: 3000-3499 kg) for sex and date of birth Controlled : Social factors	Bayley Scale of infant development II (BSID-II) = For MDI & PDI at 6 & 12 months Behaviour ratings= At 12 months Home Inventory	BSID-II: MDI & PDI are significantly lower in term-LBW compared to NBW in both 6 & 12 months of age after adjustment of socio-economic variables Behaviour ratings: Term-LBW infants were significantly less active, co-operative, vocal & happy and were more inhibited than NBW infants Home-Stimulation: Significant correlation with MDI at 6 & 12 months of age in term-LBW infants only Significant Interactions : LBW x maternal literacy on MDI & PDI Home Stimulation x LBW on MDI	BW positively correlated with cognition Controlled socio economic status Loss 36%
Indonesia Kardjati et al, 1991 Only abstract available	LBW : Birth weight <2,500 g Gestational age: Not mentioned Assessed =From birth to 12 months	Longitudinal cohort Total 561 infants from 3 Indonesian villages were grouped into LBW & NBW infants and compared .Only 9.5-12.2 % of the population was LBW	Motor development (details not available from abstract)	Motor development: No significant difference between the LBW and NBW groups	No effect of LBW on motor development Most of the LBW infants were breast fed

BW =Birth Weight; LBW=Low Birth Weight; MDI=Mental Developmental Index ; n= Number of cases; NBW= BirthWeight; PDI=Psychomotor Developmental Index; SGA= Small for gestational age

Table 1.4. Effect of SGA/term-LBW on child development and behaviour-studies from developing countries (Age: 2-15years)

Study	Definition of groups	Sample/ Study Design	Assessment Tool	Result	Comment
Guatemala Villar et al, 1984;	IUGR: Calculated if BW is <10 th percentile of a reported sex-race-specific birth weight-gestational age distribution reference.	Sub-sample from a longitudinal intervention trial compared two groups	CIS= At 6,15, 24 months	Mental score At 6 & 15 months: not significant	BW positively correlated with cognition Marked reduction in sample size at 60 months
Gorman & Pollitt 1992	Further subdivision was based on ponderal index values (PI=BW g / ht cm ³) if above or below the 10 th percentile of the PI curve of Lubchenco and colleague (1966) by gestational age standards Low ponderal index (LPI) = <10 th percentile of reference Appropriate ponderal index (API): > 10 th percentile of reference Gestational age =>37 weeks Assessed =At 6, 15, 24,36,48,60 months Follow-up =5 years (60 months)	IUGR group: n=59 Subdivisions- API-IUGR group: n=38 LPI-IUGR group1: n=21 NBW group : n=146 Controlled : Social factors and dietary supplementation	Cognitive battery of mental tests (reasoning, verbal processes, learning perceptual, analytic skills & memory) =At 36 months Preschool cognition Battery =At 60 months NBW group : n=146 Controlled : Social factors and dietary supplementation	At 24 months: Significantly lower in IUGR- API but not in IUGR-LPI Cognitive battery = Significantly lower in IUGR (NBW> IUGR LPI >IUGR-API). IUGR-API scored lower than IUGR LPI in 7 out of 8 measures at 36 months Later analyses 36 months – 60 months (Gorman & Pollitt 1992): (n=NBW/API- IUGR was 76/41 at 36 &48 months) (n=NBW/ API-IUGR was 41/19 in 5 years) In API-IUGR- Verbal ability =Significantly lower at 36 month not in 48 or 60 months Short term memory factor =Not significant at 36 & 48 month. Interaction Growth in 1 st year x SGA= significantly explained memory factor at 36 &48months. SES x SGA= significantly explained memory factor at 36months only. No effect at 60 months.	Association with maternal nutritional not observed Sample reduced markedly at later age

API=Appropriate Ponderal Index, BW =BirthWeight; IUGR = Intra Uterine Growth Restriction; LBW=Low Birth Weight; LPI =Low Ponderal Index, MDI-Mental Developmental Index; n= Number of cases; NBW= BirthWeight; PI=Ponderal Index; PDI=Psychomotor Developmental Index; SES= Socio Economic Status

Table 1.4. (continue) Effect of SGA/term-LBW on child development and behaviour-studies from developing countries (Age: 2-15years)

Study	Definition of groups	Sample/ Study Design	Assessment Tool	Result	Comment
Jamaica Gardner et al, 2003 Walker et al 2004	Term-LBW : Birth weight <2500 gram Gestational age >37 weeks Assessed =Birth, 7 months, 15 months and 24 months	As a part of a RCT, intervened term-LBW infants (control group) are compare to normal-birth-weight (NBW) Term-LBW (n = 70) NBW infants (n = 94) Controlled :Social and environmental factors	Problem solving test: Two tests,cover and support at the age of 7 months Wolke's behavior ratings: Approach, activity,emotion,vocalization and co-operation during test measured at 7 months Griffiths Scales Development was assessed at 15 and 24 months	Problem solving test: Term-LBW infants had significantly lower scores than the NBW infants in both cover (1.9 vs 2.9,p=0.003) and support test (1.6 vs 2.5,p=0.001) Behavior: Term-LBW infants vocalized less(p<0.02), & were less co-operative (p<0.001), happy (p<0.02) and active (p<0.02) than the NBW infants Total developmental quotient (DQ): Term-LBW infants had significantly lower scores than the NBW infants at the age of 15 (108.5 vs 111.7,p=0.006) and 24 months (94.2 vs 97.9, p=0.009) Subscales They also scored significantly lower in performance and locomotor subscales at 15 months and performance and hand & eye subscale at 24 months Birth measures: Ponderal index was significantly correlated with only emotion, BW with all behaviors, birth length & head circumference with most of the behaviors	BW positively associated with developmental delays,
China Jiang et al, 1991	SGA: If BW is <10 th percentile Gestational age =37-40 wks Assessed =Longitudinally from birth to 6 years	Longitudinal study Children from 1 month to 5 years, divided in to 12 age range groups. SGA (n=24) AGA (n=174) Controlled :Not controlled for any environmental factors	Brain Stem Auditory Evoked Responses: test age ranged from 1 month post-term upto 6 years	Brain stem conduction time BCT is consistently shorter in SGA infants compare to AGA infants and the difference is significant in early infancy The means of the early and late BTC interval ratio remained significantly and consistently lower in SGA infants than AGA infants after 1 year.	BW positively correlated with brain stem conduction time Small group with wide age range

BCT= Brain stem conduction time; AGA=Appropriate-for-gestational age; BW =Birth Weight; LBW=Low Birth Weight; MDI=Mental Developmental Index; n= Number of cases; NBW= Birth Weight; PDI=Psychomotor Developmental Index; SES= Socio Economic Status ; SGA= Small-for-gestational age

1.4.2. Studies on term LBW and SGA infants from developed countries

Most of the earlier studies from developed countries showed evidence indicating that term-SGA infants have more minor neurological, developmental and behavioural problems than normal birth weight children (Als et al, 1976; Fitzhardinge & Steven, 1972; Harvey et al, 1982; Parkinson et al, 1986). Whereas some studies found no significant associations or small non significant deficits (Babson & Kangas, 1969; Westwood et al 1983), earlier reviews of the development of SGA or term-LBW children showed that cognitive deficits often changed over time (Grantham-McGregor et al, 1998) and the few studies that had looked at the long term consequences had controversial findings (Hack, 1998). In this section I shall present studies of the development of term-LBW and SGA children conducted in developed countries since 1990.

Studies on early childhood

Table-1.5. presents six studies that looked for developmental outcome of term-LBW infants during infancy and early childhood (up to 5 years). One study is from the UK, one from the USA and four from Scandinavian countries. Three (Markestad et al, 1997; Andersson et al, 1997; Sommerfelt et al, 2000; 2001) of the four Scandinavian studies assessed different sub-samples of a large multicentre prospective study where 5722 women of parity one or two, who had been recruited before 20 weeks of pregnancy, were followed up.

Four studies assessed development during infancy (Roth et al, 1999; Nelson et al, 1997; Markestad et al, 1997; Andersson et al, 1997). Roth et al (1999) defined SGA as foetal weight below the 10th centile for their gestation at 3rd trimester and further classified them into SGA or IUGR. Assessment of these infants at the age of one year showed no developmental disadvantages (however no social variables or stimulation received at home were controlled here).

Two studies used the Bayley scales of infant development (BSID) - one found significantly lower mental development scores in term-SGA infants (Markestad et al, 1997) whereas the other one found significantly lower psychomotor development scores (Nelson et al, 1997) when compared to the NBW group. Markestad and colleagues (1997) also found a positive correlation of MDI with parental education and ponderal index at birth. The study by Nelson and colleague (1997) also used the Fagan Test of Infant Intelligence (FT-II), as did the study by used by Anderson and colleague (1997), but the test did not detect any developmental disadvantages in the SGA groups after controlling for social variables. However, scores on stimulation at home had a significant positive correlation with scores on FT-II in SGA infants only (Andersson et al, 1997).

The remaining two studies of this age group assessed children at the age of 4 to 5 years using different cut offs for diagnosing SGA as well as using different assessment tools. Pryor (1992) from New Zealand conducted a retrospective study by identifying 67 full term LBW infants using cut-off below <3rd centile (to minimize the chance of including a genetically small child). Forty-six NBW infants were matched on several criteria to term-SGA infants. Developmental assessment at the mean age of 4.1 years showed term-SGA infants scored significantly lower in four out of five developmental parameters and in the total score compared to NBW infants. On the other hand follow-up of a larger number of term-SGA infants (BW below 15th percentile) from the Scandinavian multicentre prospective study (Sommerfelt et al, 2000, 2001) found no significant developmental or behavioural disadvantage in these infants when compared with NBW controls.

Studies on adolescents

Table 1.6. shows nine different studies that looked for long term developmental outcome of term LBW or SGA infants from 6-19 years. Strauss and Dietz (1998) compared term LBW infants with NBW infants from the population as well as with NBW siblings to control for genetic and environmental factors.

These infants were assessed for their intelligence at the age of 7 years using the WISC and for visual motor development using the Bender Gestalt test. When these infants were compared with NBW infants from the population they showed significantly lower mental and motor scores. However many genetic and environmental factors could not be controlled at population level. In contrast, the SGA infants of the sibling cohort showed no such differences from their sibling controls. Within the LBW term group, low IQ and poor visual motor function was associated with large deficits in head circumference.

Hawdon and colleagues (1990) failed to find any disadvantage in SGA infants (BW 2nd centile on weight-for-gestational-age chart) in school achievement and intelligence compared with their NBW counterparts. However there were some positive correlations between weight-for gestational age z scores and behaviour at 10-11 years age. A study from New Zealand (Pryor et al, 1995) identified term-SGA infants based on birth weight <10th percentile from sex specific growth standards. They were assessed with serial developmental tests and showed significantly lower intelligence scores and more behaviour problems according to parents' reports at 5-11 years compared to NBW infants. Another study from Finland (Hollo et al, 2002) assessed children at the age of 10 years who were SGA at birth (BW <2.5th percentile) and compared their intelligence and behaviour with NBW infants. The findings showed significantly lower overall IQ and lower scores in short term auditory memory, perceptual organization and attention subscales in SGA infants compared to NBW infants. SGA infants also showed more neurological impairment and behaviour problems according to parents' reports. However, the study did not report about controlling for confounders and included some preterm, twin and neurologically impaired patients in the analyses. Whereas in an Australian study (O'Keeffe et al, 2003) SGA adolescents were more likely to suffer only from learning difficulties when compared with NBW (>10th percentile) adolescents, there was no difference between SGA and NBW groups in intelligence. In this study birth weight distribution was subdivided into 3 groups and compared (BW ≤ 3rd percentile, between 3-10 percentile and >10th percentile). The prevalence of learning difficulties was higher in those of birth weight < or =3rd percentile. Girls with birth weights below -3 sd had attention and reading problems.

The last four studies, two from Israel (Paz et al, 1995; 2001) and two from Sweden (Nilsson et al, 2001; Lundgren et al, 2001), looked for long term consequences of LBW/SGA babies. All were historical studies that had extracted data from 2 matched data sets. Paz and colleague (1995) reported that at the age of 17 years, term-SGA (birth weight < 3rd percentile) females had small cognitive deficits ($p < 0.03$) compared to NBW groups. SGA males had worse school achievement than those who were born at term and appropriate-for-gestational-age (AGA). This study controlled for a number of social, perinatal and birth events. Similarly, the other study (Paz et al, 2001) conducted 5 years later on a larger sample assessed both moderate term-SGA (BW < 10th percentile for gestational age) and severe SGA (BW < 3rd percentile for gestational age) subjects at the age of 17 years. In this study the author further controlled for some important maternal characteristics like maternal height, BMI, weight gain during pregnancy and smoking. The results showed significantly lower intelligence test scores (IQ) in both SGA males ($P < 0.001$) and females ($P < 0.015$). The severe SGA group had a significantly lower IQ than moderate SGA or NBW group. However academic achievement of the SGA group was not affected and was similar to the NBW groups.

Nilsson and colleague (2001), in a population based cohort study, assessed infants' developmental outcome in late adolescence across the birthweight groups (birthweight divided into decentiles). They showed a positive association between psychological assessments with increasing birth weight up to 4200 grams. However from this study it is difficult to isolate term LBW infants as the group with the lowest birth weight combined all children with weights below 2,880g. Finally, in this age group, a Swedish group (Lundgren et al, 2001) conducted a historical cohort study in which 18 year old males were assessed during military induction for intelligence and performance during stress and their birth records were retrieved. The test results were examined by birth weight and gestational age (Table 1.6.). Findings showed that participants with low birth weight, short birth length and small head circumference at birth and preterm birth were at increased risk of poorer intellectual and psychological performance than those with larger birth measures.

One study in young adults from Israel (Seidman et al, 1992) compared developmental outcomes across the range of birth weights but had no records of gestational age so is not included in the Table 1.6. It was a prospective cohort study and showed an association of later cognition and birth weight categories at ages 17 years.

Studies on young adults

I could trace six studies that investigated the long-term prognosis of term LBW or SGA infants up to 20 to 35 years of age (Table 1.7.). Sørensen et al (1997) conducted a historical cohort study in a defined region of Denmark. Men who were evaluated during induction to military service for cognitive development at 20 years of age were grouped into categories according to birth weight and gestational age (Table 1.7.). The results showed a positive association between birth weight and cognition up to a weight of 4200 g. Birth weight controlling for birth length was also associated with cognition. A French study (Larroque et al, 2001) reported increased frequency of late entry into secondary school by term SGA children (odds ratio: 2.3). In addition, a significantly higher proportion of these SGA adolescents failed to take or pass the Baccalaureate examination than the AGA adolescents (odds ratio: 1.6). Similarly, Strauss and colleagues (2000) reported a slight reduction in educational achievement in term SGA infants leading to lower professional attainment and lower income but no effect on social or emotional consequences. In a recent years a very small study from Sweden (Viggedal et al, 2004) investigated small-for-gestational age (weight <-2 sd for gestational age) children born without any complications and any signs of neurological impairment or developmental delay up to 18 months for their later neuropsychological sequelae. At the age of 21-28 years they had significantly lower IQs, specifically in verbal comprehension and figurative learning and memory functions, compared with normal controls (Table 1.7.). Finally two more recent studies looked for association of birth weight with unemployment (Kristensen et al, 2004) and depression (Gale & Martyn, 2004). Both the studies showed independent association of lower birth weight with higher risk of unemployment and depression in adult hood. Although in both studies not all infants were full term, the finding remained when preterm infants were excluded.

Studies on older adults

I located two studies (Table-1.8.) both from the UK, that followed the development of population based cohorts to over 35 years of age, and examined the effect of the whole range of birth weights.

Cheung and colleagues (2002) examined the association of BW controlling for gestational age with the Malaise Inventory score at the age of 23, 33 and 42 years in 9731 subjects. Information was available on birth measures, anthropometric measures at age 7, 11 and 16 years, maternal characteristics and socioeconomic variables. After controlling for gender, fathers' social class, parity and maternal age, psychological distress scores were inversely related to BW z scores and weight gain from birth unto the age of 7 years. An increase of one z score in BW or childhood weight gain was associated with a mean reduction in psychological distress score of 0.10 (95%CI 0.05 to 0.15) and 0.06 (0.02 to 0.10) respectively. In the other prospective cohort study, Wiles and colleagues (2005) assessed the association of depression at the age of 41-51 years with subjects' birth characteristics (birthweight and gestational age) and perinatal factors like maternal eclampsia. These subjects were participants of the Aberdeen Child Development Survey (ACDS), a cross sectional survey of learning disability in all 7 year old primary school children. Full term LBW infants had increased risk of psychological distress in later life after adjustment for potential confounders (OR=1.49, 95% CI 1.01-2.20). These findings remained unchanged after adjustment for childhood IQ and behaviour and the author suggested a direct effect of early life factors on adult mental health that was not mediated by childhood factors.

Three other studies of older adults, all from the UK (Richard et al, 2001; Thompson et al 2001; Martyn et al 1996), compared developmental outcomes across the range of birth weights. However none had records of gestational age so they are not included in the Table. One study showed an association of later cognition and birth weight categories at ages 11 to 26 years but not at 43 years (Richard et al, 2001). A second study (Thompson et al, 2001) found an association between depression at age 68 years and birth weight in

men only. In contrast, Martyn and colleagues (1996) failed to find associations between birth weight and cognition at age 48 to 70 years.

Summary of term-LBW/SGA studies in developed countries

In a recent systematic review Shenkin and colleague (2004) reported about small but consistent positive association between birth weight and childhood cognitive ability. Six studies that looked for cognition across the birthweight ranges have been reviewed. Other than those studies, I located 23 studies from developed countries since 1990 that looked for associations between term low birth weight or SGA and developmental outcome in later life. Out of them, six studies assessed development during early childhood until 5 years of age (Table-1.5.), nine studies assessed development during adolescence, between 6-19 years of age (Table-1.6.), six studies assessed development during early adulthood, between 20 to 35 years of age (Table- 1.7.) and two studies assessed development in late adulthood, after 35 years of age (Table-1.8.).

Findings

In children under 5 years the findings were inconsistent, with three of six studies finding an effect of term SGA/LBW on child development. However, three of the studies came from the same Swedish cohort and accounted for two of the ones that failed to find an effect (Anderson et al, 1997; Sommerfelt et al, 2000; 2001). The findings from studies in older subjects were impressively consistent. All the nine studies on older children or adolescents and the eight studies on adults found some effect of term SGA/LBW on at least one developmental outcome. The outcome measures varied.

In the young children infant developmental tests were used in 3 studies, Fagan's test of novelty preference in one and IQ tests in two. The infant tests showed no effect in one study (Roth et al, 1999), an effect on motor development in another study (Nelson et al, 1997), and an effect in mental development in the third study (Markestad et al, 1997). There was no effect on Fagan's test and one (Pryor, 1992) of two studies (Pryor, 1992; Sommerfelt et al 2000; 2001) using IQ tests found an effect.

In adolescents and older children eight (Strauss et al, 1998; Hawdon et al, 1990; Hollo et al, 2002; Pryor et al, 1995; O'Keeffe et al, 2003; Lundgren et al, 2001; Paz et al, 1995; 2001) of nine studies had a measure of cognition and it was affected in all seven. Four looked at behaviour and all found deficits, usually in attention and inhibition. Five studies (Hollo et al, 2002; O'Keeffe et al, 2003; Hawdon et al, 1990; Paz et al, 1995; 2001) also had a measure of school achievement or reading and four found deficits (Hollo et al, 2002; O'Keeffe et al, 2003; Paz et al, 1995; 2001). Two studies of military entrants looked at ability to work under stressful conditions (Nilsson et al, 2001; Lundgren et al, 2001) and both found deficits.

Of the eight studies in adults three looked at IQ (Sorensen et al, 1997; Viggedal et al, 2004; Wiles et al, 2005) and all found a significant deficit in SGA/LBW children but the differences were extremely small. Three studies had some measure of educational achievement (Larroque et al, 2001; Strauss et al, 2000; Viggedal et al, 2004) and two found small deficits in the SGA/LBW group. Four studies looked at psychosocial function and three found an increase in depressive symptoms (Cheung et al 2005; Wiles et al, 2005; Gale & Martyn 2004) whereas one found no effect on life satisfaction (Strauss et al, 2000). Two studies looked at employment characteristics and both found effects; one on unemployment (Kristensen et al, 2004) and one on income and holding a professional job (Strauss et al, 2000). Most of the effects were small.

Samples

Definitions of SGA/LBW group: There was variation in the type of child studied. All studies in under-five children and most of the studies in adolescents studied term SGA infants, one study looked at the range of birth weights (Nilsson et al 2001) and one looked at term LBW infants (Strauss et al 1998). Whereas of the eight studies in young and older adults, four looked at the whole range of birth weights (Cheung et al 2005, Sørensen et al 1997, Gale & Martyn 2004, Kristensen et al 2004) and three looked at SGA infants (Viggedal et al, 2004, Larroque et al 2001, Strauss et al 2000) and one looked at term LBW babies (Wiles et al 2005). However, the definition of SGA varied

and different cut-offs were used ranging from below the 3rd centile to below the 15th centile. Furthermore, different references were used.

Loss to follow up: Loss to follow up varied greatly and was generally larger the longer the follow up. In many studies loss was not always adequately reported but close scrutiny sometimes indicated large losses such as 54% (Wiles et al, 2005) and 41% (Cheung et al 20005). The effect of such losses is not clear. In young children the poorest and sickest children tend to be lost (Wariyar & Richards, 1989) and they tend to be in the SGA/LBW group thus reducing any deficit in the SGA group. However in the very long term follow up studies the subjects who have left the area are lost and they may be the more enterprising.

Confounding variables: Nearly all studies made some attempt to control for socio-economic conditions. In one study, when SGA children were compared with controls from the general population, significant deficits were found, but when siblings were used as controls there was no significant deficit (Strauss et al, 1998). This illustrates the importance of adequate controls. Many factors affect children's development and it is extremely difficult to control for all of them. Therefore it always remains possible that unmeasured poor socio-economic conditions account for at least some of the deficits found.

Finally, since the reviews by Grantham McGregor (1998) and Hack (1998) on the effects of LBW/SGA infants on later development, there have been a number of new studies of term SGA or LBW children published. Based on the above review, it can be concluded that in spite of wide variations in definition of SGA, type of outcomes assessed and different control of confounders, the findings are mostly consistent, showing an association between SGA and future intelligence, academic performance and behavioural development. The size of the effect is usually small and within normal limits and the clinical importance probably marginal.

Table 1.5. Effect of term-LBW /SGA on child development and behaviour-studies from developed countries (Age ≤ 5 years)

Study	Definition of groups	Sample Study Design	Assessment Tool	Result	Comment
UK Roth et al 1999	SGA=Estimated foetal weight <10 th centile for gestation during 3 rd trimester of pregnancy Gestational age =>36 weeks Further division on difference between repeated ultra-sound measures (1 week gap) – IUGR = If abdominal circumference > -1.5 sd scores between 2 measures SGA = If abdominal circumference < -1.5 sd scores between 2 measures Assessed = Prenatally by USG (from 3 rd trimester), at birth and 1 year	Prospective cohort study –Cases recruited from pregnancies referred for ultrasound to evaluate foetal size at 3 rd trimester Controls not referred volunteers agreed to go for repeated ultra-sound. IUGR =23 SGA = 53 Control =10 Controlled : SES not controlled	Cranial USG = after birth Neurological assessment = after birth & in first year Knobloch's developmental screening inventory = Assessed DQ for motor and adaptive sub-scales and classified if there is an impairment, at 1 year	Neurodevelopmental outcome = No difference in between term SGA & IUGR group compared to NBW Impairments = At birth of were significantly greater in combined SGA & IUGR group than that of control but at 1 year it was not significant One third cases had subtle neurological signs in SGA & IUGR groups at 1 year	No developmental risk for term SGA & IUGR group at one year Timing of intra-uterine growth restriction could not be assessed No social factors were controlled Small numbers of controls
USA Nelson et al, 1997	SGA = Based on birthweight < 15 th percentile for gestational age. Gestational age =>37 weeks Assessed =At birth and 1 year	Prospective cohort study –717 out of 949 (76%) singleton births to women followed from early pregnancy were studied over their first year of life. Controlled : For socio-demographic variables, child's growth	Bayley Scales of Infant Development : at one year age Fagan Test of Infant Intelligence (FTII) : at one year age	Bayley Scales at one year age : Mental developmental index (MDI)= No significant difference between SGA & NBW Psychomotor Development Index (PDI): Significantly lower in SGA infants. FTII =Not significant	Significantly lower PDI score in LBW infants No difference in cognitive abilities at one year in LBW infants
New Zealand Pryor, 1992	SGA = If BW<3 rd centile (to minimize the chance of including genetically small child) on weight for gestational age charts Gestational age =>37 weeks Assessed =At 2.5yrs-5yrs	Retrospective comparative study from birth records of major maternity hospital in New Zealand SGA =67 NBW (>25th centile) =46 matched for sex, birth-order, parity, ethnicity, maternal age, single/twin. Controlled : For socio-demographic variables	Stanford Binet Intelligence Scale = For separate assessments of abilities in verbal, quantitative, abstract reasoning and memory domains in addition to total score.	Intelligence Scale = SGA infants scored significantly low in 4 out of 5 developmental parameters and in total score compared to NBW infants. Important correlation : Maternal education, height and SES significantly correlated with intelligence score in total sample.	Significantly lower IQ score in term SGA

BW= Birth weight; DQ= Development quotient; IUGR= Intra uterine Growth Retardation; IQ= Intelligence Quotient; USG= Ultrasonogram; LBW= Low birth weight; MDI= Mental developmental index; NBW =Normal birth weight; PDI Psychomotor Development Index ;SES= Socioeconomic status; SGA= Small for gestational age

Table 1.5. (continue) Effect of term-LBW /SGA on child development and behaviour-studies from developed countries (Age ≤5 years)

Study	Definition of groups	Sample Study Design	Assessment Tool	Result	Comment
Norway Uppsala and Sweden Anderson et al, 1997	SGA: If BW below 15 th percentile from sex specific Norwegian growth standards, based on LMP and adjusted for parity from the Norwegian Birth Registry Gestational age=>37 weeks Assessed =7 months	Prospective cohort study Part of large multicentre prospective study where 5722 one or two parous women who had been recruited < 20 weeks pregnancy & followed up. Total 334 infants out of 436 invited were assessed. SGA=142. NBW=172 Controlled: For home environment	Fagan Test of Infant Intelligence (FT-II) = At 7 months for visual recognition memory test Home Screening Questionnaire (HSQ) –Parent administered questionnaire to measure quality of stimulation and support available at home, when the child is 13 months of age	FT-II scores = Significantly lower (57.5 vs 59.1, p<0.05) in SGA compared to NBW, which is no longer significant when home environment was controlled Home score = Significantly lower (34.3 vs 35.4, p<0.01) in SGA group compared to NBW.	No significant difference in intelligence between LBW and NBW infants Used higher cut-off for SGA diagnosis
Norway Uppsala and Sweden Markestad et al, 1997	SGA: If BW below 15 th percentile from reference standard (as above) Gestational age=>37 Further subdivision based on reference of ponderal index (PI) into- Asymmetric SGA(Low PI) = <10 th percentile of reference population Symmetric SGA (Appropriate PI)- > 10 th percentile of reference population	Prospective cohort study Part of large multicentre prospective study where 5722 one or two parous women who had been recruited < 20 weeks pregnancy & followed up. Total 835 infants were selected SGA=265 out of 357 NBW=329 out of 478 Controlled: For socio-demographic variables, parental biological factors	Bayley Scales of Infant Development 13 months	At 13 months- MDI = SGA scored significantly (112.1 vs 116.5, p<0.0001) lower compared to NBW The mean score was lower in asymmetric SGA (low PI) than symmetric SGA. PDI = Non-significant difference between two groups.	Significantly lower mental score in term SGA infants at the age of 13 months. Used higher cut-off for SGA diagnosis
Norway Uppsala Sweden Sommerfelt et al, 2000, 2001	Assessed = At 13 months SGA: If BW below 15 th percentile from reference (as above) GA=>37 weeks Assessed=At 5 years	Prospective cohort study Part of large prospective multicentre study, recruited pregnant women <20 weeks and followed up. From live births complete information was available on ~ 677 children SGA=318-338 NBW= 307-335 Controlled: For socio-demographic variables child rearing practices and maternal risk factors.	WPPSI-R = For verbal & nonverbal IQ PIC (Personality Inventory for Children) YCI (Yale Children Inventory) Raven Progressive Matrices ERS (Examiner Rating Scale) CRPR (Child Rearing Practice Report) IPE (Inventory for Parent Expression)	Child's IQ = No significant difference. Mean nonverbal IQ 4 point lower & verbal IQ 3 point lower in SGA group Child behaviour at preschool age: No significant difference between SGA and NBW infants	No significant difference in developmental measures between LBW and NBW infants Used higher cut-off for SGA diagnosis

DQ= Development quotient; IQ= Intelligence Quotient; PI= Ponderal Index; LBW= Low birth weight; NBW =Normal birth weight; SGA= Small for gestational age; WPPSI-R= Wechsler Preschool and Primary Scale of Intelligence - Revised

Table 1.6. Effect of term-LBW/ SGA on child development and behaviour: studies from developed countries (Age 6-19 years)

Study	Definition of groups	Sample Study Design	Assessment Tool	Result	Comment
USA Strauss et al, 1998	LBW: IUGR is defined by birth weight less ≤ 2500 g at term Gestational age : >37weeks Two cohort examined Population cohort: Compared LBW with NBW from the population Sibling cohort: For controlling genetic and environmental factors, compared LBW child with NBW siblings Assessed : At 7 years	Prospective cohort study enrolled mothers and children who were born from 1959-76 for National Collaborative Perinatal Project . Total 8411 women were enrolled having at least 2 pregnancies. Population cohort= Term-LBW= 2719 NBW=43,104 Sibling cohort= Term-LBW= 220 NBW=220 Controlled: For genetic and environmental factors in sibling cohort	Clinical examination and anthropometric measures = at birth WISC= For assessing intelligence at 7 years Bender Gestalt test= For assessing visual motor development	In population cohort WISC= Significantly lower IQ (6 point, $p<0.001$) in LBW infants compared to NBW Bender Gestalt test= Visual motor development significantly lower ($p<0.001$) in Term-LBW infants compared to NBW In sibling cohort- IQ not significant WISC= Not significant Bender Gestalt test= Not significant Term-LBW with head circumference ≤ 31 cm - WISC & Bender Gestalt test = Significantly lower IQ & visual motor development compared to term-LBW with head circumference >31 cm	Significant developmental disadvantages among Term-LBW infants in their behaviour and IQ when compared with NBW infants in population level, but not when compared with NBW siblings.
UK Hawdon et al, 1990	SGA : If BW<2 nd centile on weight-for-gestational-age Gestational age : 36-42 weeks Assessed & Follow-up : At birth & 10yrs-11yrs	Prospective comparative study= Boys selected from 6900 children born in and staying near Newcastle upon Tyne SGA= 30 out of 53 boys NBW= 30 matched controls with birth weight >2 nd centile on weight-for-gestational-age chart from the index child's school class Controlled: For school class, father's occupation and single parenthood	Child: Temperament Newcastle Behaviour Inventory Neurological testing WISC-R =cognitive function Young Reading test= Short form of test to assess reading attainments EPQ= Child's personality Teacher Report= CTQ= For child's classroom behaviour. Modified teacher temperament questionnaire NEQ	Intelligence & School-achievement = Not significant Behaviour: Distractibility and approachability was slightly but significantly higher in SGA infants compared to NBW Correlations: Neurotic behaviour: At home correlated significantly and inversely with GA Temperament & behaviour = Significant correlation with weight for gestational age z score (indicates 'Attention Deficit Disorder')	No developmental disadvantages in LBW infants in intelligence & school-achievement Some positive association - weight- for GA z-scores and behaviour Two extremely disabled- SGA cases were excluded.

CTQ=Conners Teacher Questionnaire; EPQ = Eysenck Personality Questionnaire; NEQ=Newcastle Educational Questionnaire ; IQ= Intelligence Quotient ;IUGR= Intra uterine Growth Retardation; LBW= Low birth weight; NBW =Normal birth weight; SGA= Small for gestational age; WISC = Wechsler Intelligence Scale for Children; WISC-R= Wechsler Intelligence Scale for Children- Revised

**Table 1.6. . (continue)Effect of term-LBW /SGA on child development and behaviour: studies from developed countries
(Age 6-19 years)**

Study	Definition of groups	Sample Study Design	Assessment Tool	Result	Comment
New Zealand Pryor et al, 1995	SGA: If birth weight <10 th percentile from sex specific growth standards. Gestational age: >37 weeks Assessed & Follow-up : At birth and every 2 years from 3-15 years of age (91% =FU at 3 yrs). Then at 18 years of age.	Prospective cohort study Part of longitudinal cohort study where ~1000 young people who were studied since birth as a part of Dunedin Multidisciplinary Health and Development Study SGA=91 NBW=932 Controlled: For socio-demographic variables	Stanford Binet Intelligence Scale =For intelligence at 5 years WISC-R =For intelligence after 5 years, every 2 yearly until 15 years Burt Reading Scores =At age 7,9,11,13,15 & 18 For behaviour Rutter antisocial score = At age 5,7,9 & 11 (parents) and at age 5,7,9 11& 13 (teachers) Revised Problem Behaviour Checklist (RPBC) =Completed by parents at age 13 & 15 years	WISC-R Intelligence test score (IQ) = Significantly lower in both SGA girls (P <0.001) & boys (P <0.04) Mean IQ for SGA is 109.0 and NBW is 101.2. Burt Reading Scores = NS but consistently lower in SGA girls. Behaviour ratings = Parents reported significantly higher scores in SGA group in 5 to 11 years than NBW group. Teachers reported no significant difference between the groups.	Significantly lower IQ in SGA at 13 years- ~8 IQ point behind than NBW & significantly worse parents reported behaviour
Finland Hollo et al, 2002	SGA : If birthweight had been <2.5th percentile on the population-based fetal growth chart. Gestational age: Mean GA 38.8 (range 27.0-42.0) weeks. 8 SGA (7%) born prematurely. 13 SGA born from twin pregnancy Assessed= 10 years	Prospective, population-based birth cohort study The SGA children born in 1985 compared with AGA children born around same time and matched for gestational age and mode of delivery. SGA= 106 children AGA=105 children Controlled: Not mentioned	WISC-R = Intelligence assessed using subscales (verbal comprehension, fine motor abilities, attention, visual perception, memory) Neurological assessment Conners' teacher rating scale & Conners' parent rating scale = for behaviour assessments Academic achievement = Included type of education, school reports & grade level	WISC-R = Significantly lower overall IQ and lower scores in short term auditory memory, perceptual organization and attention subscales in SGA than AGA infants Neurological assessment = Significantly more major neurological impairments (CP, blind, intellectually disabled) & difficulties in coordination, balances & gross motor functions in SGA than AGA infants (11 vs 3, p=0.049) Behaviour = Parents reports: SGA infants had significantly more learning problems than AGA infants Teachers report: Not significant Academic achievement =25% school failures in SGA children vs 14% of AGA children (P =0.05).	Significantly poorer academic achievement & intelligence in SGA than AGA infants at 10-year. SGA group had a few preterm infants & more twins & more neurologically impaired cases

AGA= Appropriate for gestational age; BHR= Birth weight-to-head circumference ratio; CP= Cerebral Palsy; IQ= Intelligence Quotient ; LBW= Low birth weight; n=number of subjects NBW =Normal birth weight; SGA= Small for gestational age; WISC-R= Wechsler Intelligence Scale for Children- Revised

**Table 1.6. . (continue) Effect of term-LBW /SGA on child development and behaviour: studies from developed countries
(Age 6-19 years)**

Study	Definition of groups	Sample/ Study Design	Assessment Tool	Result	Comment
Australia O'Keefe et al, 2003	SGA : Term born infants were subdivided into 3 groups based on BW- ≤3 rd percentile 3-10 percentile >10th percentile -using Australian norms for male & female infants SGA further divided into – Quartile groups on the basis of PI and BHR to measures body symmetry Gestational age : ≥ 37weeks Assessed= At birth, 6 months, 5 years & 14 years	Prospective cohort study: A total of 7388 term infants have been followed prospectively since birth. At 14 years, 5059 mothers completed reports on their child. A total of 5051 adolescents completed a Self Report, & 3703 went for psychometric tests. SGA group: ≤3 rd percentile: n~166 3-10 percentile: n~430 NBW group: >10 th percentile: n~4457 Controlled: For the level of social risk at birth.	Mothers completed child's- Child's Behaviour Checklist School progress For Adolescents- Ravens Progressive Matrices =IQ for psychometric testing Wide Range Achievement Test (WRAT) = Reading scores Youth Self Report Questionnaire (YSR) = Assessed behaviour Outcomes were compared on the basis of BW groups & measures of body symmetry	Learning difficulties= SGA adolescents in comparison to NBW infants, were more likely to experience learning difficulties. Higher prevalence was in those with birth weight < or =3rd percentile. Attentional problems= Girls of birth weight < or =3rd percentile were more likely to have attentional problems WRAT mean reading scores = NS between SGA and NBW groups. Only girls of birth weight < or =3rd percentile had low WRAT reading scores. Ravens IQ= Not significantly different Association= Body symmetry of SGA- Not significant association with later child development (cognitive ability, learning difficulties and attention control) at the age of 14 years. Severity of growth restriction- predicted later learning difficulties in both gender. Girls with severe growth restriction are at risk of attentional problems during adolescence	Modest positive association between birth weight and cognition SGA status seems to have only modest independent effects on learning, and attention in adolescence.

**Table 1.6. . (continue) Effect of term-LBW /SGA on child development and behaviour: studies from developed countries
(Age 6-19 years)**

Israel Paz et al, 1995	<p>SGA : If BW <3rd percentile for gestational age on growth curve based on whole population</p> <p>Gestational age =>37wks</p> <p>Assessed=At birth and tested prior to army conscription at age of 17 years</p> <p>Follow-up =17 years</p>	<p>Historical Prospective study by matching birth records (sex, maternal identity, birth date), of 1758 infants to the results from prior tests in army conscription at age of 17 years.</p> <p>SGA= 64</p> <p>NBW=1643</p> <p>Controlled : SES Parental education, social class, birth order, ethnic origin, birth events</p>	<p>At 17 years –</p> <p>Translated version of verbal Otis test</p> <p>Non-verbal –matrices test</p> <p>Academic achievement</p>	<p>Intelligence test score (IQ)= Significantly lower in both SGA male ($p=0.03$) & female($p<0.03$)</p> <p>Academic achievement : Significantly lower in SGA males, had more < 12 year schooling or attended vocational school</p> <p>Predictive parameter for academic achievement= Maternal education and social class</p> <p>Intra-natal events (Cesarean section & Cardio-tocographic abnormalities): had no independent effect on neurodevelopmental outcome</p>	<p>BW positively correlated with cognition.</p> <p>Information extracted from 2 data sets thus quality control and reliability not maintained</p>
Israel Paz et al, 2001	<p>SGA : If BW <10th percentile for gestational age on growth curve based on whole population</p> <p>Severe SGA if BW <3rd percentile for gestational age on growth curve based on whole population</p> <p>Gestational age =>37weeks</p> <p>Assessed=At birth and tested prior to army conscription at age of 17 years</p> <p>Follow-up = 17 years</p>	<p>Historical Prospective cohort study included 13,454 term singletons from 3 major hospitals. Data extracted from 2 database based on personal ID number= “Jerusalem Perinatal Study” & matched information from “Army Draft Board”</p> <p>SGA= 944 (tested)</p> <p>NBW=9592(tested)</p> <p>Controlled : For clinical, perinatal, and socio-demographic confounding variables in addition maternal height & BMI, weight gain during pregnancy and parental smoking</p>	<p>At 17 years –</p> <p>Translated version of verbal Otis test</p> <p>Non-verbal -Matrices test</p> <p>Academic achievement</p>	<p>Intelligence test score (IQ)= Significantly lower in both SGA male ($P < .0001$) & female ($P < .015$)</p> <p>Severe SGA had significantly lower IQ than moderate SGA or NBW.</p> <p>Academic achievements SGA was not associated with lower academic achievements compared with AGA.</p> <p>Predictive parameter for IQ= Parental education & smoking, ethnic origin, birth order, and social class. Maternal BMI had independent effect for males only</p>	<p>BW positively correlated with cognition</p> <p>Information extracted from 2 data sets thus quality control and reliability not maintained.</p> <p>On 22% cases information was missing</p>

BW= Birth weight; IQ= Intelligence Quotient; LBW= Low birth weight; NBW =Normal birth weight; SGA= Small for gestational age

**Table 1.6. . (continue) Effect of term-LBW/SGA on child development and behaviour: studies from developed countries
(Age 6-19 years)**

Study	Definition of groups	Sample Study Design	Assessment Tool	Result	Comment
Sweden Nilsson et al 2001	<p>Birth weight range used as continuous variable as well as divided into decentiles-</p> <p>≤2880 gram 2881-3130 gram 3131-3300 gram 3301-3430 gram 3431-3560 gram 3561-3680 gram 3681-3820 gram 3821-3980 gram 3981-4200 gram ≥4201 gram</p> <p>Gestational age = >37 weeks</p> <p>Assessed= 18 years</p>	<p>Retrospective cohort study -Linked birth registry data and data from an assessment of psychological function during evaluation for military service. Birth record of 90 651 young males (born 1973-1975), were obtained from the Swedish Medical Birth Register.</p> <p>They were investigated during their conscript evaluation in 1991-1994.</p>	<p>General psychological performance- for leadership evaluation</p> <p>Psychological functioning- Assessment of stress susceptibility</p>	<p>Association=A positive association was seen between birthweight and better assessment results up to a level of about 4000 g birthweight, but above that an inverse association was seen.</p> <p>Positive correlations ($P < 0.001$) were seen between psychological assessment score results and birthweight ($r = 0.07$), gestational age (0.03), head circumference (0.05), and maternal age (0.11), but inverse correlations with maternal parity (-0.11) and birth month of the offspring (-0.04).</p> <p>In multiple regression analyses, the strongest independent correlations were seen between increasing assessment scores and maternal age and birthweight (positive), as well as with maternal parity and offspring adult weight (negative).</p>	<p>A positive association between psycho- logical assessments with increasing BW up to 4200 gram. Impaired fetal growth predicts lower psycho-logical functioning & increased stress susceptibility in males during early adult life.</p> <p>Very few infants were with BW <2500 gram</p>

BW= Birth weight; NBW =Normal birth weight; SGA= Small for gestational age; WISC-R= Wechsler Intelligence Scale for Children- Revised

Table 1.6. (continue). Effect of SGA/term-LBW on child development and behaviour: studies from developed countries (Age 6-19 years)

Study	Definition of groups	Sample Study Design	Assessment Tool	Result	Comment
Sweden Lundgren 2001	<p>SGA: If < -2sd in either birth length or birth weight for gestational age and divided into 3 groups- Born short only Born light only Born short & light</p> <p>Birth weight for gestational age: divided into 3 categories based on comparison to mean BW Light for GA (<-2 sd) Appropriate weight for GA (-2 to +2 sds) Heavy for GA (>+ 2sds)</p> <p>Birth length and head circumference for gestational age divided into 3 categories analogously</p> <p>Gestational age: divided into 4 groups: Very preterm(≤31 week) Moderate preterm (32-36 week) Term (37-41 weeks) Post-term (37-41 weeks)</p> <p>Assessed: Only males were assessed at birth & at 18 years</p>	<p>Historical cohort study : This included all male singletons born without congenital malformations in Sweden from 1973 to 1978 and alive at 18 y (n =276,033). Information from the Swedish Birth Register was individually linked to the Swedish Conscrip Register. Of 254,426 conscripted males, information on intellectual and psychological performance was available for 97% and 91%, respectively</p> <p>Based on BW- Light for GA : n=6400 Appropriate wt for GA : n=233,531 Heavy for GA: n=6785</p> <p>Based on maturity- Very preterm: n=792 Moderate preterm: n=9829 Term: n=209,273 Post-term: n=27075</p>	<p>General intellectual performance (IP) : At conscription IP was measured for all men by a time limit test package covering 4 dimensions & 40 questions from each dimensions: Logical/inductive Verbal Spatial Theoretical/ technical</p> <p>Psychological performance (PP)Evaluation: using semi-structured questionnaires by specialized psychologists measured performance during stress</p>	<p>IP & PP=Low birth weight, short birth length, small head circumference at birth, and preterm birth increased the risk of subnormal intellectual and psychological performance</p> <p>Important predictor = Among SGA-born males, the most important predictor was the absence of catch-up growth. Being born SGA is associated with increased risk of subnormal intellectual scores (used standard cut off) and psychological performance.</p> <p>*The data strongly support the view that, for males born SGA, it is an advantage to have catch-up growth in length.</p>	<p>Positive association between birth measures and cognition and performance under stress</p>

GA= Gestational age; IQ= Intelligence Quotient; LBW= Low Birth weight; n= number of cases; NBW =Normal Birth Weight; SGA= Small for gestational age

Table 1.7. Effect of term-LBW/ SGA on child development and behaviour: studies from developed countries (Age 20-35 years)

Study	Definition of groups	Sample Study Design	Assessment Tool	Result	Comment
Denmark Sørensen 1997	LBW : If birth weight <2500 g Gestational age : grouped into- >37 weeks (n=3898) 33-36 weeks (n=269) <33 weeks (n=130) Assessed : At 20 years	Historical cohort study based on birth data and cognitive function evaluated during military service of 4300 Danish men at 20 years of age LBW =< 2500 g (n: 171) NBW =Grouped as follows 2501-3000 g (n= 603) 3100-3500 g (n= 1451) 3501-4000g (n=1453) 4001-4500 g (n=515) Controlled : For birth measures (birth length, gestational age) & some maternal biological factor (age, parity, marital status, education & employment)	Boerge Prien Test : of cognitive score is the number of correct answers to 78 questions and correlated with full scale intelligence quotient	Intelligence score (at 20 years of age) =Lower (39.9 vs 42.2-44.6) in LBW group compared to NBW (>2500 g -4200 g) Fetal growth seems to influence adult cognitive performance.	Positive association between birth weight and cognition up to birth weight 4200 g has functional implication Weight controlling for height related to IQ
France Larroque 2001	SGA : If birth weight and/or length <3 rd percentile for gestational age of a sex specific local standard population based growth curve Gestational age : >37wks Assessed : Participants were evaluated at a mean age of 20.6 (+/-2.1) years.	Prospective cohort study 836 full-term singletons who were born from 1971 through 1978 according to population based birth registry of Haguenau, France were identified for the study, 48% dropped. SGA = 236 full-term singletons AGA = 281 full-term, (between the 25 th and 75 th percentiles) Controlled : Parental socioeconomic status, family size, gender maternal age and education	Schooling age = entry into secondary school (normal age: 11 years) Examination at the end of secondary school : normal age: 18 years	Schooling age =Late entry into secondary school was more frequent for the SGA than the AGA children (odds ratio: 2.3) Examination at the end of secondary school =A significantly higher proportion of term SGA adolescents failed to take or pass the baccalaureate examination than AGA adolescents (odds ratio: 1.6).	Positive association between birth weight and age of enrolment and achievement in school Being born SGA at term is associated with poorer school performance at 12 and 18 years.

AGA=Appropriate for Gestational Age; **IQ**= Intelligence Quotient; **LBW**= Low Birth Weight; **n**= number of cases; **NBW** =Normal Birth Weight; **SGA**= Small for gestational age

Table 1.7. (continue) Effect of term-LBW/ SGA on child development and behaviour: studies from developed countries (Age 20-35 years)

Study	Definition of LBW	Sample Study Design	Assessment Tool	Result	Comment
UK Strauss et al, 2000	SGA : If birth weight < 5 th percentile for age at term from British reference standards. Gestational age : > 37 wks Assessed & Follow-up =5,10,16 26 yrs tested 93%, 80%, 72% & 53% of recruited samples	Prospective cohort study A total of 14189 full term infants were studied as a part of British birth cohort-1970. SGA =1064 NBW = 13125 Controlled : For social class, sex, region of birth, and presence of fetal or neonatal distress.	School performance and achievement = At 5,10 & 16 years Years of education, occupation, income marital status, life satisfaction, disability and height = At 26 years Teachers' Report =completed questionnaire concerning child's academic achievement and difficulties	Academic achievements = Small but significantly lower in SGA infants at 5, 10 and 16 years of age. Years of education, employment, hour of work, marital status, life satisfaction = NS at 26 years, but SGA infants less likely to get professional job ($p<0.01$) and had lower income ($p<0.01$) compared to NBW Teachers' Report =Less likely to rate SGA infants compared to NBW (13% vs 20%, $p<0.01$) in the top 15 th percentile and more likely to refer for special education (4.9% vs 2.3%, $p<0.01$) at 16 years	Significantly lower academic achievements and professional attainments, but no long term social or emotional consequences
Sweden Viggedal, 2004	SGA = If birth weight <2 standard deviation for gestational age Gestational age = >37 weeks (only 2 cases <37 weeks but > 36 weeks) Assessed = 5,10,18 months then between 21-28 years	Prospective cohort study enrolled 17 SGA children who were born at Sahlgren Hospital and followed until 18 months. Healthy NBW healthy child without resuscitation and normal APGAR score, enrolled from same hospital from birth register SGA = 12 cases (at 24 years) out of 17 NBW =18 controls (at 24 years) out of 30	Comprehensive neuropsychological assessment : of the main aspects of cognitive function includes intelligence, verbal functions, visuo-spatial and visuo-constructive functions, learning and memory of words and figures, attention, lateral preference, motor functions, and cognitive adaptive functions were investigated. Educational achievement : From detailed questions	Intelligence quotients : Significantly lower IQ score in SGA group, specifically in verbal comprehension and figurative learning and memory functions, compared with normal controls. Educational achievement and social adjustment : No differences between the groups.	Positive association between birth weight and cognition Very small sample size

IQ= Intelligence Quotient; LBW= Low Birth weight; n= number of cases; NBW =Normal Birth Weight; SGA= Small for gestational age IQ; WRAT =Wide Range Achievement Test

Table 1.7. . (continue) Effect of term-LBW/ SGA on child development and behaviour: studies from developed countries (Age 20-35 years)

Study	Definition of groups	Sample Study Design	Assessment Tool	Result	Comment																		
UK Gale & Martyn , 2004	<p>LBW or SGA not defined= Birthweight was categorized as ≤2.5 kg 2.51-3.00 kg 3.01-3.50 kg >3.5 kg</p> <p>Gestational age = grouped into five categories <37 weeks 37-39 weeks 40-420weeks >40 weeks</p> <p>Assessed= At birth, 16 years & 26 years</p>	<p>Prospective cohort study Study examined the relation between BW & risk of psychological distress and depression. Total 11622 out of 16500 surviving participants, who participated in the 1970 British Cohort Study (BBC) , responded at 16 years follow-up.</p> <p>Total number of subjects in each BW categories at the age of 26 years-</p> <table><tr><td></td><td>Women</td><td>Men</td></tr><tr><td>Total :</td><td>n=4547</td><td>n=3745</td></tr><tr><td>≤2.50 kg:</td><td>n=270</td><td>n=594</td></tr><tr><td>2.51 to 3.00 kg:</td><td>n=959</td><td>n=1380</td></tr><tr><td>3.01 to 3.50 kg:</td><td>n=1895</td><td>n=19,441</td></tr><tr><td>>3.5 kg:</td><td>n=1423</td><td>n=1576</td></tr></table> <p>Controlled: For socioeconomic condition, maternal depression, Early separation from the mother, parental marital disruption, & experience of local authority care.</p>		Women	Men	Total :	n=4547	n=3745	≤2.50 kg:	n=270	n=594	2.51 to 3.00 kg:	n=959	n=1380	3.01 to 3.50 kg:	n=1895	n=19,441	>3.5 kg:	n=1423	n=1576	<p>General Health Questionnaire = A 12-item questionnaire to assess psychological distress at age 16 years (n=5187 out of 16500 surviving members of (BBC)</p> <p>Malaise Inventory =to assess depression at age 26 years (n=8292)</p> <p>History of depression =Information taken about history of depression at age 26 years (n=8292)</p>	<p>Malaise Inventory =In total 1574 (19%) subjects scored ≥7, indicating depression. Depression was more in females than males (23% vs 14.1%)</p> <p>Women whose BW was <3 kg had an increased risk of depression at age 26 years (OR=1.3; 95% CI 1.0-1.5) compared with those who weighed >3.5 kg.</p> <p>In men there was a trend towards increasing risk of depression with decreasing BW</p> <p>History of depression=No significant linear trend of between birth weight and history of depression in either sex.</p> <p>However LBW men (≤2.5 kg) had a trend to report more history of depression at age 26 years (OR=1.6, 95% CI 1.1-2.3) compared with men of NBW.</p>	<p>Birthweight associated with later depression though not always significant</p> <p>Impaired neuro-development during foetal life may increase susceptibility to depression</p> <p>Psychiatric morbidity was based on self – completion scales</p>
	Women	Men																					
Total :	n=4547	n=3745																					
≤2.50 kg:	n=270	n=594																					
2.51 to 3.00 kg:	n=959	n=1380																					
3.01 to 3.50 kg:	n=1895	n=19,441																					
>3.5 kg:	n=1423	n=1576																					

CI= Confidence interval; IQ= Intelligence Quotient; LBW= Low Birth weight; n= number of cases; NBW =Normal Birth Weight; OR= Odds Ratio; SGA= Small for gestational age

Table 1.7. (continue) Effect of term-LBW/ SGA on child development and behaviour: studies from developed countries (Age 20-35 years)

Study	Definition of LBW	Sample Study Design	Assessment Tool	Result	Comment																																	
Norway Kristensen et al, 2004	<p>No definition for SGA BW was standardized for gender in order to compare BW categories between genders.</p> <p>Mean BW±SD- Girls: 3571± 531 gram Boys: 3443 ±512 gram</p> <p>Gestational age = categorised as <33, 33-36, 37-39, 40-42, 43-46 weeks groups</p> <p>Assessed= 29 years</p>	<p>Prospective cohort study Through linkage between several national registers containing personal information on birth, education and employment. Study participants were all 308 829 singletons born in Norway in 1967-1971 as registered by the Medical Birth Registry of Norway who were national residents at age 29.</p> <p>BW z score categories-</p> <table><thead><tr><th></th><th>Women</th><th>Men</th></tr></thead><tbody><tr><td>Total :</td><td>n=150803</td><td>n=158026</td></tr><tr><td>≥3.00:</td><td>n=327</td><td>n=308</td></tr><tr><td>2.00 to 2.99:</td><td>n=2961</td><td>n=2921</td></tr><tr><td>1.00 to 1.99:</td><td>n=18,708</td><td>n=19,441</td></tr><tr><td>0.00 to 0.99:</td><td>n=54,249</td><td>n=57,420</td></tr><tr><td>-0.01 to -1.00:</td><td>n=53454</td><td>n=55985</td></tr><tr><td>-1.01 to -2.00:</td><td>n=16,981</td><td>n=17,440</td></tr><tr><td>-2.01 to -3.00:</td><td>n=3066</td><td>n=3419</td></tr><tr><td>-3.01 to -4.00:</td><td>n=864</td><td>n=892</td></tr><tr><td><-4.00 :</td><td>n=193</td><td>n=200</td></tr></tbody></table> <p>Controlled: For social disadvantage, gestational age, birth order and childhood disease</p>		Women	Men	Total :	n=150803	n=158026	≥3.00:	n=327	n=308	2.00 to 2.99:	n=2961	n=2921	1.00 to 1.99:	n=18,708	n=19,441	0.00 to 0.99:	n=54,249	n=57,420	-0.01 to -1.00:	n=53454	n=55985	-1.01 to -2.00:	n=16,981	n=17,440	-2.01 to -3.00:	n=3066	n=3419	-3.01 to -4.00:	n=864	n=892	<-4.00 :	n=193	n=200	<p>Unemployment: Defined as a lack of personal income & having no education in the calendar year of their 29th birthday as registered by the National Insurance Administration and Statistics Norway.</p>	<p>Unemployment:BW below the standardized mean was associated with unemployment. The risk of unemployment increased by decreasing BW for both gender and also after adjustment for potential confounding factors. The association was evident both in people with or without social disadvantage, as well as people with or without childhood disease. Excluding preterm birth did not change the relationship</p> <p>BW below the standardized mean explained much less of the unemployment risk than did social disadvantage (attributable fractions 8.0% versus 28.3% for women and 10.0% versus 40.2% for men).</p>	<p>Positive independent association between BW & unemployment at age 29, also in the normal birth weight range controlling for gestational age.</p>
	Women	Men																																				
Total :	n=150803	n=158026																																				
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Table 1.8. Effect of term-LBW /SGA on child development and behaviour-studies from developed countries (Age >35 years)

Study	Definition of groups	Sample Study Design	Assessment Tool	Result	Comment
UK Cheun g et al, 2005	IUGR = BW was standardizing for GA separately for each sex giving a birthweight SD Score or z score (a unit increase in BW z score is an increase in 1 SD birth weight for GA Assessed =At ages 7, 11, 16 years for anthropometry and SES only. At ages 23, 33, 42 years for depression scale.	Population based birth cohort study = 1958 birth-cohort followed to age 42 years. Total 9731 participants had valid perinatal, postnatal, and adult data extracted from medical records. Subjects assessed- Age of test men (n) women (n) 23 years 4212 4240 33 years 3728 3809 42 years 3780 3889 Controlled: For gender, father's social class, parity & maternal age	Malaise inventory scores = Measured psychosomatic symptoms at ages 23, 33, and 42 years.	Psychological distress score = Inversely related to BW z score and weight gain from birth to the age of 7 years. A unit increase in BW z score or childhood weight gain was associated with a mean reduction in psychological distress score of 0.10 (95%CI 0.05 to 0.15) and 0.06 (CI 0.02 to 0.10), respectively. BW & weight gain were also inversely related to the odds of having a high level of psychological distress, with odds ratios being 0.90 (CI 0.85 to 0.95) and 0.93 (CI 0.89 to 0.98), respectively.	Association (inverse) between psycho-logical health in adults and BW & growth in early childhood.
UK Wiles et al, 2005	Defined as = LBW/NBW based on BW (if <≥ 2.5kg or 5.5 lb) & term/preterm based on gestational age in weeks The groups were -Full term NBW (n= 5058) -Preterm NBW (n=248) -Full term LBW (n=143) -Preterm LBW (n=123) Assessed = At birth, 7 years & at the age of 41-51 years	Prospective cohort study = Participants were of Aberdeen Child Development Survey (ACDS) a cross sectional survey of learning disability in all primary school children born between 1950-1956 & had data on BW for GA from Aberdeen Maternal & Neonatal Data bank (AMND, 1948). They were routinely assessed for IQ test at 7 years in school. At age 41-51 years, 51.9% of initial participants (n=5572,) were traced & assessed for psychological distress. Controlled: For gender, father's social class, parity & maternal age	Learning disability = Assessed on all primary school children (ACDS) IQ tests (Moray House picture Intelligence Test) = All school children at 7 years Behaviour rating (Rutter Scale B) = Teachers report on all school children at 7 years Psychological distress = Using 4 item of 12 item General Health Questionnaire (GHQ)	IQ & childhood behaviour: LBW was associated with increased odds of cognitive deficits (IQ<100) and childhood behavioural disorder in both term (OR 2.12 & 1.96 respectively) and preterm (OR 1.20 & 2.10 respectively) infants, at the age of 7 years Adult psychological distress : Full term- LBW infants had increased risk of psychological distress in later life after adjustment for potential confounders (OR=1.49, 95% CI 1.01-2.20). Findings remained unchanged after adjustment for childhood IQ and behaviour. A 1 s.d. decrease in BW for gestational age was associated with a 4% increased odds of psychological distress in adulthood (OR=1.04, 95% CI 0.97-1.12).	Positive association between BW (particularly full term LBW infants) and psychological distress in later life. This was not mediated by childhood factors, suggesting a direct effect of early life factors on adult mental health. A neurodevelopmental pathway may therefore be implicated.

BW= Birth weight; CI= confidence interval; GA= Gestational Age; IQ= Intelligence Quotient; IUGR= Intra uterine growth retardation; LBW= Low Birth weight; NBW
=Normal Birth Weight; SGA= Small for gestational age

1.5. Nutrition during pregnancy

In this section I shall discuss the published literature on nutrition during pregnancy under the following sub headings:

- Food supplementation during pregnancy
- Micronutrients during pregnancy

1.5.1. Food during pregnancy

The effect of protein-energy supplementation (food) in undernourished pregnant women on improving birth weight is moderately well established and has been replicated in several studies (Prentice et al, 1983; Mora et al, 1979a; McDonald et al, 1981; Lechtig et al, 1975; Ceesay et al, 1997). However the effect on birth size varied among studies and was not always significant (de Onis 1998, Merialdi et al, 2003). In a Cochrane review of 13 trials with protein energy supplementation of pregnant women, Kramer and Kakuma (2003) showed a modest increment of maternal weight gain, mean birth weight and slight reduction in risk of LBW. On the other hand only limited data are available on the effects on child development.

There is considerable concern that the deficiency of energy and protein during pregnancy may detrimentally affect not only birth weight but also the neurodevelopment of the child. A review of animal research findings relating malnutrition to brain structure shows that certain brain structures such as the cerebrum, the hippocampus and the cerebellum are particularly sensitive to nutrition during intrauterine life. Nutritional deficiencies during early life can cause permanent alteration of the cerebrum, the hippocampus and the cerebellum in animals that is associated with impaired functional outcomes (behaviour, cognition) in later life (Levitsky & Strupp, 1995). Thus maternal nutrition might have an important role in foetal brain development in humans. However, direct evidence linking the two is not available. Several observational studies suggested a beneficial role of maternal diet during pregnancy on birth size and gestational age (Bendich, 2001; Torf et al, 1998; Keen & Zidenberg-Cherr, 1994; Jeans et al, 1955) and child development (Kirksey et al, 1991; Chavez et al, 1998).

However, because under-nutrition is usually associated with conditions of poverty that also affect children's mental development, experimental studies with supplements are required to demonstrate causal relationships.

In this section I will discuss the literature on the relationship of nutrition during pregnancy and subsequent neuro-development to the offspring.

Search criteria: To access the relevant literature I carried out a MEDLINE search using PubMed. The search criteria were restricted to nutrition (protein-energy) during pregnancy and its association with later neurological or developmental outcome in human-subjects. I considered the following developmental outcomes: cognition, motor development and behaviour during childhood. As some studies looked for associations of food during pregnancy with psycho-educational and psychiatric changes in young adults, I also included them.

The details of these studies that linked maternal nutrition and child development are summarized in Tables 1.9.-1.13. First I shall describe the observational studies, then the food deprivation studies (due to Dutch Famine) during pregnancy, followed by supplementation studies. For the latter, first I shall describe the studies that supplemented mothers alone followed by studies that supplemented both mother-infant pairs.

Observational studies

In one observational study in Egypt (Table 1.9.) early pregnancy weight and maternal intake of animal-source foods were significantly associated with orientation and habituation behaviour in neonates along with birth weight (Kirksey et al, 1991). This behaviour indicates the ability of information processing in infants and predicts later cognitive development (Colombo, 1993). However the study was biased by mothers' recall. The population was from relatively good socio-economic backgrounds and the mothers were not malnourished with mean BMI \pm SD was 25.2 ± 4.0 .

In another observational study in Mexico (Chavez et al, 1998) a group of 82 mother–child pairs were studied. The psychomotor development of the young infants was correlated with nutritional status of mothers during pregnancy as well as nutritional status of the child itself. Mothers were observed from the 5th month of pregnancy until the child was 6 months of age. Infants were assessed by the Neonatal Brazelton test and Bayley Scale of Infant Development (BSID) at 3 and 6 months (Chavez et al, 1998). There was a positive correlation of mother’s weight and skin folds thickness with neonatal behaviour and psychomotor development. Maternal consumption of animal source foods and fruits correlated positively with the child’s development and corn tortillas and beans correlated negatively.

Famine studies

The occurrence of famine (Table 1.10.) provided a quasi-experimental situation. The German army blocked food supplies to some cities in the Netherlands from October 1944 to May 1945. Subsequent food rationing caused restricted food supplies mainly to major urban cities with populations of more than 40,000. The supply first resulted in moderate (official food rations, 4200-6300 kJ/d) then severe (<4200 kJ/d, lowest up to 450 kJ/d) nutritional deficiency. The cities in the west were affected most and referred to as “famine regions”. The other cities in the north and south suffered less seriously and were considered as the “non-famine regions”. In the non-famine regions, official food supply never fell below 1300 kJ/d. Subjects who were exposed to the Dutch famine in utero were investigated in adulthood in different settings.

Table 1.10. shows studies where young Dutch men were given a developmental and psychiatric examination during military induction. They were classified by the degree and timing of their prenatal exposure to nutritional deficiency based on their birth date and birthplace. Here the exposed group is compared with the unexposed group. This comparison was unique in many ways because, firstly, the whole community was exposed to an acute crisis of nutritional insult during an extremely precise time period. Secondly, this externally produced famine minimized the bias of wide ranging social factors that usually confound the association between maternal nutrition and child development in usual peacetime situations. Thirdly, some comparison areas which were not affected by food restrictions during the same period were available.

Fourthly, large numbers of same aged adults were assessed during military induction and birth records of most of them were available. The results showed significantly increased risk of antisocial behaviour (Neugebauer et al, 1999) and schizoid personality disorders (Hoek et al, 1996) in adult males whose mothers had been exposed to severe prenatal nutritional deprivation during the 1st and 2nd trimesters. However, no effect of this acute prenatal food deprivation was observed on cognitive development in adulthood (Stein et al, 1972).

This suggests that acute nutritional insults to the developing brain in utero might affect certain behaviours in later life but not cognition, but these findings cannot be generalized as here well-nourished Dutch mothers were exposed to acute food deprivation. The situation is different in developing countries where chronic malnutrition is prevalent. In the Dutch famine, maternal prepregnancy energy reserves might have acted as a physiological buffer to the deficits and protected the children's cognitive functions. Finally there is a possibility of under reporting of the true problem where only the adult males were examined.

Table 1.10. shows other sets of studies in the offspring of the famine victims. All the famine-exposed population was compared with a non-exposed (control) population for the numbers who developed major affective disorder and schizophrenia requiring hospitalization. Diagnosis and assessment was done according to international classification of disease (ICD) and records in the Dutch national psychiatric registry. Brown et al (2000) compared major affective disorders that required hospitalization in exposed (severely food deprived <1000 kcal/d over a trimester) and control birth groups (had food ration \geq 1000 kcal/d throughout pregnancy). The exposed group was again divided into 3 subgroups based on the trimester that experienced severe food deprivation and all were selected from 6 large cities of western Holland with populations of more than 40,000. The results showed a significantly higher risk ($p < 0.001$) of major depression in subjects whose mothers were exposed to severe nutritional deprivation at the 2nd and 3rd trimesters when compared to the control group. In this same sample, Susser et al (1996) observed a two-fold statistically significant increase in the risk for schizophrenia in both men and women of the exposed group compared to the control group.

In an earlier report Susser and Lin (1992) examined whether acute food deprivation during the first trimester was a risk factor for schizophrenia in both famine and non-famine regions. Their findings showed that in the famine region there was a substantial increase in the number of women who were hospitalized with schizophrenia (Relative risk is >2) in the group exposed to severe intrauterine food restriction (<4200 kJ/d) during the 1st trimester, but not for men and not in the moderate food deprived (4200-6300 kJ/d) group.

In the non-famine region there was no severely food-deprived group, however the birth cohort exposed to moderate food deprivation during the 1st trimester showed a trend of increased risk of schizophrenia for women. This suggests that intrauterine nutritional deprivation can also cause psychiatric problems in later life and that might be gender-specific.

Supplementation of mothers only

Pencharz et al (1983) carried out a supplementation study in which school aged children born between 1962-1970 whose mothers were supplemented during pregnancy by the Montreal Diet Dispensary (MDD) were examined for school performance (Table 1.11.). They were compared with children of mothers delivered in the same hospital and matched retrospectively for several characteristics. They were also compared with the next older or next younger siblings born between 1960-1972 when the mothers received no prenatal supplement by MDD. Findings showed a slight benefit from prenatal supplementation on birth weight but no additional effect on school performance. However this study suffered from a big loss of subjects and the supplemented group had more married mothers than the unsupplemented group.

Another early study in the USA (Osofsky, 1975) used time lagged controls and compared the offspring of mothers who received high protein (80.3 g/d) supplementation during pregnancy with those of unsupplemented mothers (Table 1.11.). The control group was recruited prior to the recruitment of the supplemented group.

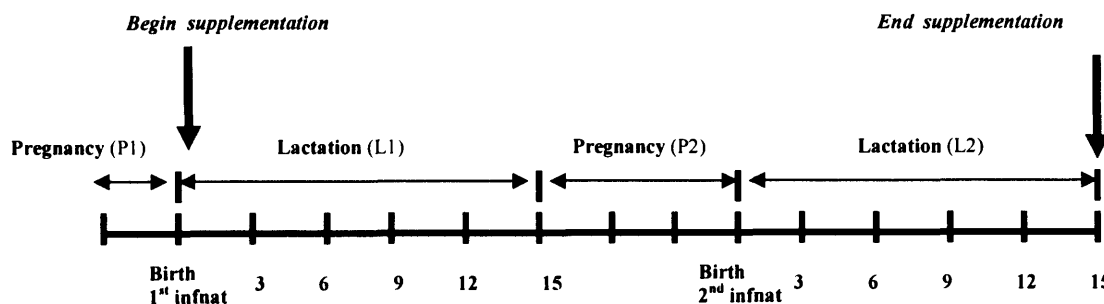
The detailed dietary history of the control group was recorded and their mean \pm SD protein intake was 71.3 \pm 26.6 g/day and calorie intake was 1919 \pm 665 cal/day. But the amount of supplement prescribed to the supplement group was not mentioned. The subjects were from poor income families and the racial backgrounds of the groups were not detailed. However 92% of mothers in the control group were black. At enrolment the control mothers had significantly fewer years in schooling ($p<0.05$), lower occupation level ($p<0.01$) and lower hemoglobin level ($p<0.05$). The neonates were assessed for behaviour using the Brazelton Behavioural Scale on ~3rd day of age along with other clinical assessments. The infants in the supplemented group had significantly smaller birth size and a lower Apgar score than the control group. They were also significantly less active and less reactive on the Brazelton measures compared to the control group. However interpretation of this study is difficult for several reasons. Firstly, though the mothers were from poor socio-economic status, they were not a truly nutritionally deprived group and their dietary intake approximated their daily recommended requirements. Secondly, results were not controlled for the significant group differences between supplemented and controlled groups. Finally, it is not clear whether being less active and less reactive of the supplemented group is a good or bad sign as these infants were not followed further.

Table 1.12. presents two further nutritional intervention studies where only the mothers were supplemented. One study conducted in the United States (Rush et al, 1980) supplemented 814 poor black urban pregnant mothers with protein, calorie and multiple micronutrients. The population was randomized into three groups - high protein and calorie group, low protein and calorie group and control group. All three groups received multiple micronutrients (>17 micronutrients). The infants were measured at birth for somatic and neurological measures and at 1 year for psychological measures including Bayley mental and motor test, object permanence test, free play and visual habituation. The infants in the high protein-calorie (40 g+ 470 kcal) supplemented group showed lowered birth weight (not statistically significant) and higher preterm delivery and neonatal death (approaching significance) compared to the control, whereas the balanced protein-calorie (6 g protein +322 kcal) diet showed a non significant increment in birth weight and a significantly ($p<0.05$) lowered rate of preterm delivery and neonatal death.

The study also failed to find benefits of prenatal supplementation on motor and mental development of the offspring assessed by the Bayley scale. Only the high protein supplemented group scored significantly higher in three developmental measures (habituation, dishabituation and length of play episodes) that had no significant association with growth related measures, birth weight or its predictors and age of testing. However, the population was unlikely to be undernourished and the control group received multiple micronutrients of varying dosage.

In Taiwan (Joos et al, 1983; Hsueh & Meyer, 1981; Adair & Pollitt, 1985) a double blind randomised trial of supplementation of rural mothers was conducted beginning soon after birth of one infant and continuing through the next pregnancy and the period of lactation that followed for up to 15 months (Figure 1.8.).

Figure 1.8. Taiwan study design



Becon Chow Study Design (Adopted from Adair and Pollitt, 1985)

The population had no severe malnutrition and the supplementation (group A) contained a high content of calorie and protein. The control group (group B) received a placebo containing very low calories from sweeteners but both groups received 18 micronutrients. The children of the first and second pregnancy were examined for growth and had developmental assessments at 8 months using the Bayley scale and IQ at 5 years using the Stanford Binet Intelligence Scale. Only 60 of the first pregnancy infants (A1 and B1) could be tested for developmental measures at varying test-age due to the late addition of developmental assessments to the study protocol.

All the mothers had a first study child (A1 or B1 group) who received supplementation or placebo throughout the lactation period and a second study child (A2 or B2 group) who received supplementation or placebo both pre and postnatally. Two sets of comparisons were made. The supplemented were compared with the non-supplemented according to the intention to treat analysis comparing the A1 with B1 and A2 with B2. In addition, the first study children (A1 & B1) were compared with the second study children (A2 & B2) of the same mothers. At the age of 8 months developmental analyses focused on the second study child only (Joos et al, 1983). Birth weights of the second male infants (A2) were significantly higher at 162 grams in the supplemented groups than those of the first male infants (A1) of same mothers, but there was no difference between the first (B1) and the second (B2) children in non-supplemented groups. Infants of supplemented mothers during pregnancy and lactation (A2) had significantly better motor development at the age of 8 months compared to the infants of non-supplemented mothers (B2). However there was no difference in mental development. At the age of 5 years, both the groups did not differ in IQ in within or between group comparisons. Sex of the offspring was found to be an important mediator of the effects of supplementation.

Supplementation to both mother and child

Table 1.13 shows 4 studies in rural Guatemala, Mexico, Colombia and rural Louisiana in the USA. All these studies gave food supplementation to both mothers (pregnancy and lactation) and their children (infancy and early childhood) and found benefits.

In Guatemala a supplementation study (1969-1977) with long-term follow-up (1988-89) was carried out in two pairs of rural villages. The study assessed the effect nutritional supplementation had during pregnancy and early childhood on growth and mental development of children. The villages were randomised to one of the two dietary supplements, high calorie with protein (Atole) and low calorie with no protein (Fresco) which were given to pregnant women and children up to 7 years in special feeding centres. The subjects were self selected and were allowed to have the supplement *ad libitum* (Martorell et al, 1995). Within each supplement group, calories showed consistent positive association with birth weight (Lechtig et al, 1975) and the percentage of LBW was 9% vs 19% in the mothers receiving high

caloric supplement ($\geq 20,000$ cal) vs low caloric supplement ($\geq 20,000$ cal). Mean birth weight was also significantly ($p < 0.025$) higher in the high caloric-group but there was no significant difference between caloric and protein-caloric supplement Ted groups (Lechtig et al, 1975). However birth weight was available for only 62% ($n=405$) of births and the total amount of supplement (calories and protein) consumed by the mother was related to the duration of pregnancy. Developmental assessments were conducted using a “cognitive infant scale” at the age of 6, 15 and 24 months; thereafter a “preschool battery” was used yearly up to 7 years of age. The language subscale showed the strongest association with supplementation, however the memory and perception subscale also showed a significant positive correlation with supplementation. In general, developmental outcome suggested “causal linkage between nutrition and cognition competence” (Freeman et al, 1980).

In a separate analysis, Barrett and colleagues (1982; 1985) compared the behaviour of children at 6-8 years of age who received high supplementation with those who received low supplementation according to the level of maternal supplementation during pregnancy and child supplementation from birth to 4 years. The children's behaviour was observed in a range of structured and unstructured situations. The findings showed that children in the high supplementation group were more exploratory, more persistent, more involved and active, less anxious and presented with more affective expression (both positive and negative). However, the initial randomized study design was broken in this method of data analysis.

Because the original analysis did not use an intention to treat approach, the analyses were repeated by Pollitt et al (1993; 1995) who maintained the original design. The results were published in a monograph. Subjects chosen for the analyses had been supplemented prenatally and during at least the first two years of postnatal life. The results showed children in the Atole villages performed better in motor skills at 24 months than children in the Fresco villages. They also scored better on tests of perceptual organisation and verbal ability at age 4 and 5 years after controlling for sex, age and attendance. Supplements were most beneficial to children of low socio-economic status and the interaction between treatment x socio-economic status (SES) was significant at age 4 & 5 years. Long term follow up showed that adolescents of the Atole group scored significantly higher than the Fresco group on numeracy,

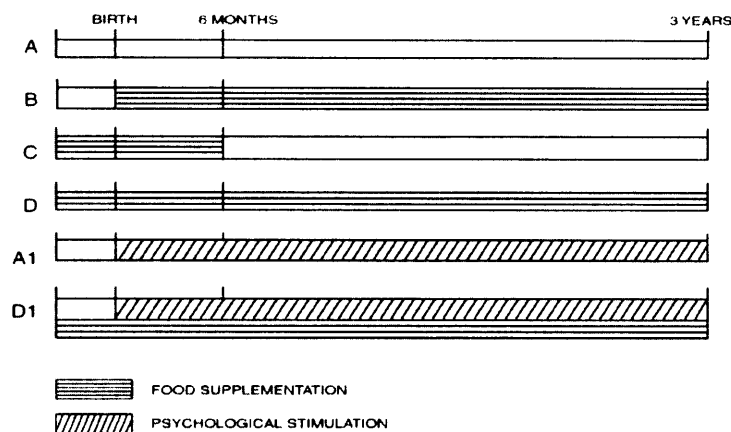
knowledge, reading and vocabulary and there was a treatment x SES interaction for all of the above tests. In addition, there was a treatment x maximum grade reached interaction showing that supplementation was particularly beneficial to those with more schooling.

The longitudinal Mexican study (Chavez & Martinez, 1975; 1982; 1994) was conducted in a small poor village and focused on the effect of nutritional intervention on children's growth and development. A small group of pregnant mothers (n=17) were supplemented from ~ 45 days of pregnancy through lactation and their children were supplemented until 10 years of age. The children were supplemented from ~4 months of age. The effect of supplementation was compared with an equal number of non-supplemented mothers and their offspring who were enrolled two years earlier (Chavez and Martinez, 1982; 1975). Birth weight was 180 g higher in the supplemented group than the control group and low birth weight was 7.3% and 36.9% respectively in the supplemented and control group (Chavez and Martinez, 1982). Developmentally the supplemented children were found to be more active, exploratory and sociable. They were carried, wrapped up and cradled less during the day time (Chavez and Martinez, 1975). The children of supplemented mothers also scored higher on the Gesell scale when assessed from 3 to 24 months of age compared to control children. In addition they performed better at primary school (Chavez and Martinez, 1982). At the age of 18 years, only supplemented boys scored higher when assessed by Raven's Matrices (Chavez et al, 1994).

In the Colombian study (Vuori et al, 1979; Waber et al, 1981; Mora et al, 1981) the effect of "nutritional supplementation" and/or "home education for infant stimulation" was evaluated (Figure 1.9.). Low income families with at least one malnourished child were enrolled. Four hundred and fifty six pregnant women in the 1st or 2nd trimesters were randomly assigned to one of six intervention groups. The 1st group (Group A) was the control and received no intervention. The 2nd group (Group B) was supplemented from 6 months of age up to 3 years. The 3rd group (Group C) was supplemented during the 3rd trimester of pregnancy until the child was 6 months of age and the 4th group (Group D) supplemented for the whole study period (that is from the 3rd trimester of pregnancy until the child's 3rd year). The 5th group (Group A1) was enrolled in the maternal education programme from birth to 3

years but received no supplementation. Finally the 6th group (Group D1) received both maternal education and supplementation throughout the study period. The whole family received food to minimize the sharing of food among the family members. Compliance was not monitored. Supplemented infants were less irritable and less likely to cry on being removed from the nipple at 15 days than the non-supplemented group. They were also less apathetic than the non-supplemented group at 4 months of age (Mora et al, 1979b). In addition supplemented children habituated significantly more frequently and moved less frequently during visual habituation test at 15 month of age compared to non supplemented children (Vuori et al, 1979).

Figure 1.9. Intervention schedule for the experimental groups in Bogota, Colombia.



Children's developmental quotients (DQ) were measured every 2 months from 4-6 months, then 6 monthly up to 3 years. All supplemented groups except group C (from the 3rd trimester up to 6 months posnatally) performed better than the controls. Supplementation up to 3 years of age (groups B, D & D1) was found to have a significant benefit in all subscales of the Griffiths test and total DQ (Waber et al, 1981). The nutritional intervention mainly influenced performance on a subset of items that were primarily motoric whereas maternal education programmes improved language performance (Waber et al, 1981). Follow up at age 6-7 years showed that supplementation up to 36 months benefited scores on reading readiness. Intervention

showed no benefit on arithmetic or knowledge. However the only published report of this follow-up was an abstract (Super et al, 1991).

Hicks et al (1982) in Louisiana assessed the effect of a special supplemental food programme “Women, Infants and Children (WIC)” to the low income pregnant women and preschool children on subsequent child development and behaviour of the children. The study compared early supplementation (starting from the prenatal period) vs late supplementation (starting after the 1st year of the child) of children. Twenty-one sibling pairs (19 black and 2 white) were enrolled in the study. The younger sibling received WIC benefits prenatally from the 3rd trimester until 12 months (at least) of life, while the older sibling enrolled in the programme after the 1st year of age. At follow-up, the mean age of the younger siblings was 6.3 years and the older siblings was 8.8 years when the children were assessed for intelligence, school performance and behaviour. Results indicated significant enhancement of intelligence and behavioural measures in early supplemented sibling compared to late supplemented one. However developmental assessments could not have been blind and selection bias was also there as the older sibling was recruited into the programme after the initial screening for being nutritionally at risk. Perhaps the main problem was that the groups were assessed at different ages.

Summary of food during pregnancy studies

All together I identified 11 studies that looked for an association between maternal nutrition and child development. Of these 2 were observational, one was on the effects of exposure to famine and 8 were supplementation studies.

Both the observational studies (Kirksey et al, 1991; Chavez et al, 1998) found that the intake of animal food during pregnancy was associated with child development. However these types of studies may be biased by maternal recall and accurate measurement of dietary intake is very difficult in community settings. Moreover, dietary intake is likely to be confounded by socioeconomic background.

The acute food deprivation during the Dutch famine was an unusual situation and could not be generalized to developing countries. The Dutch pregnant women had good prepregnancy weight that would have been somewhat protective for the foetus

(Schieve et al, 2000) so not comparable to the condition of chronic malnutrition usually found in women in developing countries. The association of severe food deprivation during the early trimesters with increased risk of antisocial behaviour, highlights the importance of adequate nutrition during pregnancy, even in mothers with good nutritional status. However the role of psychological stress on mothers due to a war situation and famine cannot be ruled out.

Out of the 8 supplementation studies, 4 supplemented mothers alone (Pencharz et al, 1983; Osofsky et al, 1975; Rush et al, 1980; Joos et al, 1983) and 4 supplemented both mother and infants (Chavez & Martinez, 1975; Pollitt et al, 1993; Waber et al, 1981; Hicks et al, 1982).

Among the 4 studies that only supplemented mothers, 3 found no benefit from food supplementation (Pencharz et al, 1983; Osofsky, 1975; Rush et al, 1980). These studies were all from developed countries and it is not clear from the published documents to what extent the mothers were nutritionally vulnerable. In addition, two supplementation studies in the USA and Canada were not randomized and compared children of supplemented mothers with matched controls (Pencharz et al, 1983) or time lagged controls (Osofsky, 1975) and found no clear benefit from supplementation. However Pencharz's study lost a large number of subjects and Osofsky's study had a control group which was not well matched. Perhaps anthropometrically the mothers were not malnourished and the all the infants were of normal birth weight (>3000 g).

Two other studies that supplemented mothers only were RCTs and conducted in New York (Rush et al. 1980) and rural Taiwan (Joos et al, 1983). In New York, though the selected mothers weighed less than 140 pounds at conception, the mean weight was much higher than that of rural mothers (101 ± 12 pounds at 6.8 months pregnancy) in a developing country like Bangladesh (Faveau & Chakraborty, 1994). However, these mothers were from poor low-income families with protein intakes of <50 g/day. Similarly the only maternal supplementation study from a developing country (Joos et al, 1983) addressed a population which was poorly nourished with low protein intakes (< 40 g/day) and a mean maternal weight of 110 pounds with BMI 20.7 kg/m² at enrolment. This population somewhat represented the population of

developing countries and the treatment showed a benefit in motor development at the age of 8 months, but not in IQ at 8 months or 5 years. They also showed no harmful effect of prenatal 40g/day protein supplementation whereas mothers in the New York study showed increased preterm delivery, higher LBW and more neonatal deaths in the group supplemented with the same amount of protein. In the New York study the children in the high protein group also showed no developmental benefit when compared to those in the low protein group. This information highlights the different response to similar supplementation in different populations and also the importance of targeting high risk groups for supplementation. In spite of the probability that malnourished mothers are likely to deliver developmentally vulnerable children, not a single study targeted malnourished mothers for supplementation trials to observe the development of their offspring.

Among the 4 supplementation studies that supplemented both mother and infants, 3 were from developing countries (Chavez & Martinez, 1975; Pollitt et al, 1993; Lechtig et al, 1975; Waber et al, 1981) and one was from a developed country (Hicks et al., 1982). All 4 studies showed some sort of developmental benefit in the children. However the effect of supplementation during pregnancy alone could not be assessed here separately. Only the Bogota study (Waber et al, 1981) had a group of pregnant mothers who were supplemented from the third trimester of pregnancy until the first 6 months after delivery and who showed a slight developmental benefit at 6 months but not at 36 months, indicating that benefits were not sustained.

The maximum benefit was observed in the group who were supplemented both pre and postnatally up to 36 months of age. In addition, methodological problems were also associated with all these studies. In the Mexican study (Chavez & Martinez, 1975) the group assignment was not random and used historical control. The US study (Hicks et al, 1982) had small sample sizes and the groups were assessed at different ages. In the Guatemalan study (Pollitt et al, 1993; Lechtig, 1975) the original design had specified 3 pairs of villages but, for budgetary constraints, the less accessible pair had to be dropped, thus reducing the statistical power (Martorell 1995). The assigned villages were also not identical in terms of parental occupation, literacy and child's quality of schooling.

Finally, use of varying amounts of multiple micronutrients by the Taiwan, New York and Guatemalan (Table 1.14) studies made it impossible to separate the effect of food from that of micronutrients as there might have been an interaction between them. No studies of supplementation in pregnancy targeted malnourished mothers with BMI < 18.5 kg/m² and observed developmental effects on their infants. In our study we planned to examine the effect of supplementation on malnourished mothers separately from the better nourished ones.

Thus the debate on the topic “whether brain development in utero is resistant to maternal under nutrition” remains unresolved. In studies where mothers were supplemented and children benefited, it is difficult to determine whether supplementation directly caused that effect or the effect was mediated through maternal wellbeing or better caretaking behaviour. From the above discussion it is apparent that to date, no supplementation trial on mothers targeted a high risk group and it is difficult to draw causal inferences from trials that supplemented both mother and children. Thus there is an urgent need for well designed RCTs with nutritional supplements on vulnerable groups to evaluate both the infants’ nutritional and developmental sequelae.

Table 1.9. Observational studies during pregnancy showing association between dietary intake and growth and development of the offspring

Study	Study design & exposure	Sample	Measures	Results	Comment
Egypt Kirksey et al, 1991	Observational pregnancy cohort study- linked development of offspring with maternal dietary intake Maternal food intake history- Information on individual food and beverage intake in 2 consecutive days /month from 3 rd month of pregnancy until delivery. Assessed: 5 th month of pregnancy until 7days postnatally	121 out of 312 pregnant mothers, aged 15-45 years, enrolled at <4 month pregnancy and continued until delivery. Maternal anthropometry: Maternal weight (end of 1 st trimester): mean 61.0±10.3 kg Maternal height: mean 155.3±5 cm Pregnancy weight-gain (3-9 month): mean 7.7± 3.2 kg	Dietary intakes - History taken using self reported questionnaire. Food sample weighed by trained dietitian. Anthropometry Mothers-, skin fold thickness, height and weight Infants- Birth weight, Length, Dubowitz Assessments Gestational age Behavioural assessment Within 7 day a using – BNAS	Early Pregnancy weight gain- Significantly related to 4 items of orientation and behaviour Early pregnancy weight & BMI- Significantly related to infants self quieting and response to examiner Gestational age- Positively correlated with several orientation items, reflexes and irritability Maternal food intake during 3-9 months- Positive associated between energy intake from animal source and habituation behaviour (cluster of 4 behaviour items) of neonates	Positive association- between maternal nutrition and neonatal behaviour. Biased by mothers recall.
Mexico Chavez & Martinez 1998 (Abstract only- article is in Spanish)	Observational study- Correlated child development with maternal dietary intake, nutritional status and child's own nutritional status Maternal food intake history food intake from 5 th month of pregnancy until delivery. Assessed: 5 th month of pregnancy until 6 months age of infant	82 mother child pair observed from 5 th month of pregnancy until 6 months of child's age Maternal anthropometry: Not mentioned	Dietary intakes Anthropometry- Maternal skin fold thickness, birth measures BNAS: At Birth BSID: At child's 6 month	Maternal weight and skin folds thickness- Positive correlation of with neonatal behaviour and psychomotor development. Maternal consumption of animal – food and fruits were correlated positively and corn tortillas and beans correlated negatively with child's development	Positive association- between maternal nutrition & neonatal behaviour

BNAS=Brazelton Neonatal Behaviour Assessment Scale; BSID= Bayley Scale of Infant Development; cm= Centimeter; kg= Kilogram

Table 1.10. Association of food deprivation of pregnant women during “Dutch Hunger Winter” with neuro-behaviour of offspring in adulthood

Study	Study Design & exposure	Sample	Measures	Results	Comment
Dutch Hunger Winter From October 1944 to May 1945 Neugebauer et al, 1999 Hoek et al, 1996	Retrospective cohort study. Comparison between exposed (varying degree) and non-exposed across regions Famine region- Severe deficiency = food ration <4200 KJ/d (less than 1000 kcal 14-22 g protein, 114-144 g carbohydrate & 12-28 g fat) Moderate deficiency= food ration 4200-6300 KJ/d (1000-1500 kcal, 37-42 g protein, 212-247 g carbohydrate & 24-41 g fat) No deficiency = food ration > 6300 KJ/d Non-famine region- Moderate deficiency= food ration 4200-6300 KJ/d No deficiency = food ration > 6300 KJ/d	Total 100543 Dutch men of age ~18 years were classified by the degree and timing of their prenatal exposure to nutritional deficiency based on their birth date and birthplace. Famine region- Severe deficiency: n=14310 Moderate deficiency: n=9615 No deficiency: n=45,007 Non-famine region- Moderate deficiency: n=21094 No deficiency: n=10517	Diagnosis of ASPD (Neugebauer, 1999) By psychiatric interview at time of medical examination for military induction, using the International Classification of Diseases, Sixth Revision (International classification of disease-ICD-6) Diagnosis of schizoid personality disorder (Hoek, 1996)	Prenatal exposure to severe(< 4200 KJ) maternal nutritional deficiency during pregnancy: ASPD: risk is significantly greater (OR =2.5) in men born to mothers exposed to severe deficiency during 1 st and/or 2 nd trimesters but not in 3 rd trimester. Prenatal exposure to moderate deficiency was not associated (Neugebauer, 1999) Schizoid personality disorder: risk is significantly greater (OR =2.01) in the men born to mothers exposed to severe nutritional deficiency (Hoek, 1996)	Positive association Between nutritional deprivation & later antisocial behaviours of the offspring Study finding could not be generalized to developing countries Selection bias, only males were assessed
Dutch Hunger Winter From October 1944 to May 1945 Stein et al, 1972	Retrospective cohort study. Comparison between exposed and non-exposed across regions Exposed group= From 7 famine stricken cities of the west Holland Control group= From 11 not affected cities of south, east and north of the country. Assessed at the age of ~19 years, who were given psychiatric examinations for military induction	Retrospective birth cohort based on date and place of birth. 125000 males, aged 19 years, born in selected cities from 1st January 1944 to 31 st December 1946. Exposed group: n=20,000 Control group: n=105,000	Intellectual performances: Severe mental retardation: clinically diagnosed as (idiot/ imbecile/ mongoloid) Mild mental retardation: clinically diagnosed as ‘debilitas mentis’ I Q: Assessed by Raven Progressive Matrices (Dutch Version)	Performance of ~19 years adult males whose mothers starved during pregnancy compared to non starved: Severe mental retardation: Non significant difference Mild mental retardation: Non significant difference Intelligence quotient: Non significant difference and had no clear association with changing level of mean birth weight	No association Study finding could not be generalized to developing countries Selection bias, only males were assessed.

ASPD= Anti Social Personality Disorder; g=Gram; KJ/d= Kilo Joule /day; kcal= Kilo-calorie; n=Number of population; OR= Odds Ratio

Table 1.10. (Continued) Association of food deprivation of pregnant women during “Dutch Hunger Winter” with neuro-behaviour of offspring in adulthood

Study	Study design and exposure	Sample	Measures	Results	Comment
Dutch Hunger Winter From 1944 to 1946 Brown et al, 2000	Retrospective cohort study. Exposed group: Births occurred to women exposed to mean intake of < 1000 Kcal / day over a trimester Subgroup- Exposed during 1 st trimester Exposed during 2 nd trimester Exposed during 3 rd trimester Control group: Births occurred to women exposed to mean intake of ≥1000 Kcal / day through-out pregnancy Assessed at the age of ~18 years.	Birth cohort – Aged 18 years men and women born to mothers who had prenatal exposure to Dutch famine, residing in 6 big cities of western Holland Exposed group - 1 st trimester: n=9656 2 nd trimester: n=14,645 3 rd trimester: n= 17,668 Control group: n= 115,877	Major affective disorder: Assessed by international classification of disease ICD-9, from Dutch national psychiatric registry (records from 1970-1996). Diagnostic subtypes are- Unipolar affective disorder : Manic depressive psychosis- depressed type Bipolar affective disorder: Manic depressive psychosis - manic type - circular type but currently manic - circular type but currently depressed - circular type, mixed	Major affective disorder (required hospitalization) – Risk is significantly high (p <0.001) for subjects whose mothers were exposed to nutritional deprivation at 2 nd and 3 rd trimester – compared to unexposed group. By sex: Men = Significantly higher relative risk for 2 nd and 3 rd trimester exposed group Women= Significantly higher relative risk for 3 rd trimester exposed group	Positive association of intrauterine nutritional insults to major affective disorder in later life Study finding could not be generalized to developing countries
Dutch Hunger Winter From 1944 to 1946 Susser et al , 1996	Retrospective cohort study. Exposed group: Births occurred to women exposed to mean intake of < 1000 kcal / day over a trimester Subgroup- Exposed during 1 st trimester Exposed during 2 nd trimester Exposed during 3 rd trimester Control group: Births occurred to women exposed to mean intake of ≥1000 Kcal / day through-out pregnancy Assessed at the age of 24-48 years	Birth cohort – Aged 24-48 years men and women born to mothers who had prenatal exposure to Dutch famine, residing in 6 big cities of western Holland Exposed group - 1 st trimester: n=9656 2 nd trimester: n=14,645 3 rd trimester: n= 17,668 Control group: n= 115,877	Schizophrenia: Hospital records reviewed and frequency of patients with schizophrenia was identified based on documentation from national psychiatric registry	Birth cohort exposed at the height of the famine- showed a 2 fold statistically significant increase in the risk for schizophrenia (RR=2) in both men (RR=1.9) and women (RR=2.2)	Positive association of intrauterine nutritional deprivation and schizophrenia in later life Study finding could not be generalized to developing countries

kcal= Kilo-calorie; n=Number of population; RR= Relative Risk

Table 1.10. (Continued) Association of food deprivation of pregnant women during “Dutch Hunger Winter” with neuro-behaviour of offspring in adulthood

Study	Study design and exposure	Sample	Measures	Results	Comment
Dutch Hunger Winter From October 1944 to May 1992 Susser et al , (Abstract only)	<p>Retrospective cohort study. Comparison of hospitalized schizophrenia cases from prenatally famine exposed and non-exposed groups (only during first trimester) in the famine affected regions</p> <p>Severe deficiency = food ration <4200 KJ/d (less than 1000 kcal 14-22 g protein, 114-144 g carbohydrate & 12-28 g fat)</p> <p>Moderate deficiency= food ration 4200-6300 kJ/d (1000-1500 kcal, 37-42 g protein, 212-247 g carbohydrate & 24-41 g fat)</p> <p>No deficiency = food ration > 6300 KJ/d</p> <p>Assessed at the adult age</p>	Only first trimester food deprived group who were hospitalized for schizophrenia	Hospital records reviewed for Schizophrenia. It was diagnosed based on documentation from national psychiatric registry.	<p>Exposure to severe nutritional deficiency</p> <p>During 1st trimester- Substantial increase in hospitalized schizophrenia for women (RR is >2), but not for men.</p>	<p>Positive association between intrauterine nutritional insults and schizophrenia in later life that requires hospitalization</p> <p>Study finding could not be generalized to developing countries.</p>

g=Gram; KJ/d= Kilo Joule /day; kcal= Kilo-calorie; RR= Relative Risk

Table 1.11. Non-randomized studies of supplementation of pregnancy on child development and school performance of the offspring

Study	Study design	Sample	Measures	Results	Comment
Montreal, Canada Pencharz et al, 1983	Matched-control supplementation trial Study group : School children (10-15 years) born between 1962-1970 , during when the mothers were prenatally supplemented with whole milk, egg, oranges and iron-folate tablet Control group (matched): Children of non-supplemented mothers, retrospectively matched for language group, parity, weight at conception, trimester of registration for care and year of delivery Control siblings: Other siblings , next older and next younger siblings born between 1960-1972 , during when mothers were not supplemented prenatally Assessed: at birth and at the age of 10-15 years	Supplemented pregnant mothers received nutritional supplements by Montreal Diet Dispensary Maternal anthropometry: Not mentioned Study group: n=406 Control group: n=286 Control sibling: n=153	Anthropometry- Birth measures School performance of the children= Language and mathematical achievements	Birth weight= small (40 g) but significant increment of birth-weight in the supplemented group School performance of the children= Not significant difference	No benefit on later school performance 39% of original sample could be traced and tested
Philadelphia Osofsky, 1975	Time lagged-control supplementation trial Study group : High protein (80.3 g/day) nutritional supplement during pregnancy Control group: Non-supplemented control group was recruited prior to the recruitment of the supplemented group. Their daily protein intake was 71.3±26.6 g/day and calorie intake was 1919±665 cal/day Assessed: Around 3 rd day after birth	Low income pregnant women (<28 week pregnancy) recruited from Philadelphia Temple University Hospital Maternal anthropometry – Weight (onset of pregnancy): 131 pounds(60 kg) Height: not mentioned Pregnancy weight-gain (through-out pregnancy) : 27 pound (12 kg) Study group: n=118 Control group: n=122	Anthropometry: Birth measures BNAS : Neonatal behaviour assessment	Anthropometry =birth weight, length, head size. BNAS= Lower score in - response decrement to pin prick, amount of activity at peak excitement, amount of startling, ascend from quiet to agitated state. Higher score in - general tonus & inanimate auditory orientation	Unclear association Groups were not comparable

BNAS=Brazelton Neonatal Behaviour Assessment Scale; cal= Calorie; g=gram; kg= Kilogram; n= number of subject ; NS= Not Significant

Table 1.12. Study of supplementation to mothers only during and after pregnancy on birth-outcome and later child development

Study	Design /Supplement	Sample	Measures	Results	Comment
Taiwan Becon Chow Study	RCT Supplement group (A) = High Cal (800 kcal/d) + Protein (40g/d)+ 18 MN* (Subgroup A1 =child born before & A2 =child born after supplementation) Placebo group (B) = placebo of 6 kcal/d for 1 st 4 yrs then 80 kcal for adding sweeteners +18 MN* (Subgroup B1 =child born before & B2 = child born after placebo treatment) Supplementation =within 3 weeks after birth of 1 st infant until 15 months after delivery of 2 nd infant Assessed = Upto 5 years of age	Subjects: Rural pregnant mothers with low protein intake (<40g) but no frank malnutrition, having at least one normal male child and planned to have more children 225 out of 294 gave birth to 2 children; 69 dropped (24%) Maternal anthropometry – Prepregnancy weight : ~49 kg Height: ~155 cm Pregnancy weight-gain (mean±sd) : 7.51±2.8 kg) Supplement group A : n=99 Placebo group B : n=99	Anthropometry – At birth-Birth weight , length, head & chest circumference, triceps & sub- scapular skin fold thickness, At 8 month Bayley Scale (research version) : Mental (10 items) and motor (7 items) score at 8 months of age (Joos et al, 1983) Stanford Binet Scale: Intelligence at 5 years of age (Hsueh et al, 1981)	BW – No between group (A vs B) differences. In supplemented group 2nd (A2) male infants was significantly higher than the 1 st male infants (A1) of same mothers. Growth- Between males significant intra-group differences (A2>A1) in weight from birth to 6 months and head circumference from birth to 12 months MDI at 8 mo –NS (between and within group) PDI at 8mo- Significantly higher (p=0.05) in supplemented group compared than placebo group (A2 > B2) IQ at 5yrs- No significant difference between and within the groups	No treatment effect of intention to treat analysis on birth weight but beneficial effect on motor development at 8 months
New York City. Rush et al, 1980	RCT Supplement group- high protein 40 g+ 470 cal + 18 MN* Complement group- balance protein 6 g+ 322cal + 17MN* Control group –only 16 MN* Duration =soon after enrolment (<30 wk pregnancy) until delivery Assessed = Upto 5 years of age	Subjects: 814 Poor black urban pregnant mothers out of 1051 enrolled Supplement group (n=263) Complement (n=272) Control group (n=279) Inclusion Pre- pregnancy-weight <110 lb low pregnancy-weight gain Having at least one previous LBW child Protein intake <50 g/d	Child At Birth Anthropometry – Birth weight , length, head & chest circumference, triceps & sub-scapular skin fold thickness, APGAR score, Gestational age Clinical prognosis Neurological exams Child at 1 year- Anthropometry Bayley mental/motor Object permanence test Free play Visual habituation (habituation, dishabituation, length of episodes)	Maturity: Mature infants were not different between the groups. Premature infants were significantly lower in supplemented group than non supplemented group Birth weight: Among heavy smokers it is significantly higher in supplemented group than non-supplemented group. Among mothers with previous LBW infant it is significantly lower in supplemented group than non-supplemented group At 1 year- Difference among groups Anthropometry =Non significant difference Bayley score & free play = Non significant difference Visual habituation = Supplemented group did significantly better (3 tests)	Negative effect of pre-natal nutrition supplement on BW Improvement in 3 psycho-logical measures not mediated via foetal growth. Amount of MN varied among groups

* see composition on Table.1.14.

BW= Birth weight; LBW= Low birth weight; MN= Multiple micronutrients; RCT= Randomised control trial; n= number of subjects

Table 1.13. Food supplementation during and after pregnancy and children to assess effect on birth-outcome and child development

Study	Design and supplement	Sample	Measures	Results	Comment
Mexico Chavez et al, 1975, 1981, 1982	Interventional study. Supplemented group: Mothers=Half skimmed milk (64 g/day, 400 kcal) during pregnancy from 24 th week of pregnancy. Then whole milk (600 kcal) during lactation. Infants (from 3-4 months)=Milk (180 ml) and strained food ad libitum . Control group: non-supplemented pregnant mothers, enrolled 2 years prior to supplemented mothers	2 groups of 17 mother-child dyads-supplemented vs non-supplemented in previous year (Chavez et al, 1975, 81,82)	Anthropometry Weight, Length/height, at birth, during infancy and childhood for children. Maternal measures also taken during pregnancy. Gesell tests of development = 3-24 months Behaviour assessments (Chavez et al, 1975, 81,82)	Birth weight: 180 g higher in supplemented group compared to control group LBW %: supplement group-7.3% control group 36.9 % IQ= Higher score in children of supplemented group compared to children of control group Behaviour= Children of supplemented group were more active, exploratory and sociable compared to children of control group. They moved 6 times as much as non-supplemented groups and refused to be carried or wrapped up	Positive association Developmental test could not be blinded. Very small sample Match criteria not clear. Six case-control pairs were sibs thus possibility of food sharing is there
USA Rural Louisiana Hicks, 1982	Early supplement group: Children (mean age 6.3 year) who received nutritional supplements prenatally from 3 rd trimester of pregnancy until 1 year age. Late supplement group: Older siblings (mean age 8.8 yr) who received no pre-natal supplements but supplemented after 1 year of age	Comparison between siblings, both received special supplemental food programme for women and infants and children (WIC) Study group: n=21 Control group: n=21	Anthropometry WISC-R for IQ, Attention span Visuomotor synthesis chool performance school grade-point average Behaviour	Early supplement group: Anthropometry = Significantly greater ht/age WISC-R = Significantly higher score on digit span, draw-a-person, full scale IQ Visual-motor synthesis = Significantly higher Attention span = Significantly higher School performance- school grade-point average= Significantly higher	Positive effect of pre- and post-natal supplements on BW & development Non blinded cognitive tests Older siblings screened for supplementation

BW= Birth weight;g/day= Gram/ day; IQ= Intelligence Quotient; kcal= kilo calorie LBW= Low birth weight; NBW =Normal birth weight; WISC-R= Wechsler IntelligenceScale for Children-Revised

Table 1.13. (continue) Food supplementation during and after pregnancy and children to assess effect on birth-outcome and child development

Study	Design and supplement	Sample	Measures	Results	Comment
Bogota Vuori et al, 1979; Waber et al, 1981; Mora et al, 1981	<p>RCT</p> <p>Supplement enriched bread, dry skimmed milk and cooking oil for whole family</p> <p>Every HH member-> 1yr (623 kcal+20 g protein)</p> <p>Infant weaned < 6 mo- Whole dry milk 1 lb/wk if age < 2 months & 2 lb/wk if age > 2 months</p> <p>Pregnant +lactating women-856 cal+38.4 g protein + vitamin & mineral tablets (composition not mentioned).</p> <p>Supplemented infants -If 3-6months old, 125 g/wk of high protein milk + vegetable mix+18 mg FeSO₄. From 6mo-1 yr, 1 lb milk+ 250 g of high protein vegetable mix+ 37.5 FeSO₄+ vit A 200000 IU/6 month</p> <p>Randomized to 6 groups-</p> <p>Group A - control</p> <p>Group B- supplement from 6 months to 3 year</p> <p>Group C - supplement during the 3rd trimester of pregnancy until child's 1st 6 months</p> <p>Group D- supplement 3rd trimester of pregnancy until child's 3rd year (whole study period).</p> <p>Group A1 - enrolled in a maternal education programme (stimulation- Maternal education, Infant education curriculum of "High Scope Foundation") without any supplement</p> <p>Group D1- Supplemented and stimulated throughout study period.</p>	<p>433 families randomized to 6 groups.</p> <p>Visual habituation tested on- Supplemented (144) vs un-supplemented (100) infants</p> <p>Griffiths test & Corman-Escalona Einstein scale tested on- Group A (n=54)</p> <p>Group B (n=60)</p> <p>Group C (n=57)</p> <p>Group D (n=57)</p> <p>Group A1 (n=34)</p> <p>Group D1 (n=42)</p>	<p>184 children completed all measures</p> <p>Anthropometry: At birth (weight, length, head size)</p> <p>Visual habituation: At 15 days: by checkerboard</p> <p>Griffiths test: At 4, 6, 12, 18, 24, and 36 months of age:</p> <p>Corman-Escalona Einstein scale : At 4, 6, 12, 18 months to measure cognitive competence</p> <p>Cognitive Battery: At 36 months: (Perceptual analysis, short term memory, language)</p>	<p>BW: Significantly increased (50 g) in male child of supplemented group.</p> <p>Maternal weight gain had +ve association with BW</p> <p>visual habituation : Un-supplemented infants showed less initial attention followed by slower habituation and higher levels of movement than the supplemented infants</p> <p>Griffith's test –Supplemented Children performed better than un-supplemented. Rx effects were more pronounced for girls than for boys.</p> <p>Cognitive Battery Significant improvement in perceptual analysis in supplement group</p> <p>Language performance + Perceptual analysis improved by maternal education programme</p>	<p>Positive effect of pre- and post-natal supplementation on birth measures and child development.</p> <p>Difficult to isolate the impact of prenatal supplementation alone</p> <p>Compliance not monitored</p>

BW= birth weight; FeSO₄= ferrous-sulphate; HH= House hold; kcal= kilocalorie; IU=International unit; lb= pound; lb/wk= pound /week; g/wk = gram/ week; n= number of subjects

Table 1.13. (continue) Food supplementation during and after pregnancy and children to assess effect on birth-outcome and child development

Study	Design, Supplement	Sample	Measures	Results	Comment
Guatemala INCAP study Pollitt, 1993, 1995 Freeman, 1980 Martorell, 1995 Lecthig, 1975	RCT Pregnant Mothers + infants + young children of 4 villages. were supplemented ed libitum with - Atole (high calorie & protein) in two villages- Calorie 163 kcal/682 kJ + 11.5 g protein + 9 MN* per cup or 180 mL) Or Fresco (low calorie & no protein) in two villages - Calorie 59 kcal/247 kJ + 0 g protein + 7MN* /cup). Duration : RCT: 7 years (1969-1977) and cross sectional follow-up:(1988-1989)	Self selected subjects: Total children from 4 combined villages who were 3359 exposed to supplement "atole" or "fresco" Live births in supplemented group=671 Maternal Wt (~12 wk)=49 kg Ht=149 cm BMI=22 kg/m ²	Anthropometry Weight, Length/height, 6 circumferences and skin fold thicknesses at birth, during infancy and childhood for children. Maternal measures also taken during pregnancy. Preschool Battery -language, short term memory, perceptual analysis & cognitive composite Raven's Standard Progressive Matrices for Intelligence At adolescence battery of Psycho-educational & information processing tests	Birth weight: Positive association of maternal weight gain and dietary intake with birth weight Among children(<7 yrs) Preschool Battery Significant improvement in language & cognitive composite measure »IQ: Atole benefited motor but not mental development at 24 months and perceptual organization and verbal skills at age 4 & 5 years but not 3 and 6 years At age 13-29 years Psycho-educational & information processing tests) - Atole group scored significantly higher 4/6 of the tests -knowledge, innumeracy, reading and vocabulary and 2/7 information processing tests-memory and paired associates than Fresco. Behaviour: Children receiving high levels of supplementation were more exploratory, more persistent, more involved and active, less anxious and displayed more affective expression. SES: Children of low SES benefited most	Positive effect of pre- and post-natal supplementation on birth weight and child development.

* see composition on Table.1.14.

BW= Birth weight; g/wk = gram/ week; IU=International unit; kcal= kilocalorie; KJ= Kilo-Joule; lb= pound; lb/wk= pound /week;LBW= Low birth weight; MN= Multiple micronutrients; n= number of subjects RCT= Randomised control trial; n= number of subjects; SES =Socio economic condition

Table.1.14. Amount of micronutrients used in Different Pregnancy Interventions along with food supplementation

Micronutrients	NewYork (8 oz can of beverage with added riboflavin ,as a marker, given 2can /day and control group given 1 capsule)			Taiwan (One12.5 oz can ē chocolate flavored drink given 2 times/day)		Rural Guatemala(INCAP) Fruit Flavored drink180 ml/cup		
	Supplement	High protein group	Complement	Control	Supplement	Placebo	Atole	Fresco
				No protein group	Gp A	Gp B	>4 mo	From 69-71
Vit A (Units)	6000(IU)	4000(IU)	4000(IU)	4000(IU)	2500 UPS	-	0.4	0.5
Vit D(Units)	400	400	400	400	200 UPS	-	-	-
Ascorbic acid (Vit C) mg	60	60	60	60	37.5	-	-	-
Thiamine (B1) mg	3.0	3.0	3.0	3.0	0.8	-	0.4,0	4.0
Riboflavin (B2) mg	15.0	15.0	15.0	2.0	0.9	-	0.1	1.1
Niacinamide mg	15.0	15.0	15.0	10.0	10.0	-	0.5	1.5
Pyridoxine (B6) mg	2.5	3.0	3.0	3.0	0.8	-	0.3	18.5
Cyanocobalamin (B12) µg	8.0	3.0	3.0	3.0	1.0	-	-	-
Vit E (Units)	30(USPU)	-	-	-	5.0 (IU)	-	-	-
Biotin µg	200.0	Not analysed	Not analysed	-	-	-	-	-
Calcium (Ca) g	10.0	2.5	2.5	2.5	0.5	-	0.4	-
Magnesium (Mg) mg	100.0	12.0	12.0	0.15	-	-	-	-
Phosphorus (P) g	-	-	-	-	-	-	-	-
Iron (Fe) mg	60.0	40.0	40.0	117	0.4	-	0.3	-
Folic Acid (FA) µg	350.0	350.0	350.0	350.0	6.0	-	0.2	5.0
Fluride mg	-	-	-	-	-	-	-	-
Zinc (Zn) mg	4.0	0.084	0.084	0.085	-	-	0.02	0.2
Selenium (Se) µg	-	-	-	-	-	-	-	-
Iodine (I) µg	150.0	100.0	100.0	100.0	-	-	-	-
Ca pantothenate mg	1.0	1.0	1.0	1.0	4.0	-	-	-
Sodium (Na) g	-	-	-	-	0.2	-	-	-
Pottassium (K) g	-	-	-	-	0.9	-	-	-
Copper (Cu) mg	2.0	0.15	0.15	0.15	0.5	-	-	-
Manganese (Mn) mg	-	-	-	-	1.0	-	-	-
Fibre g	-	-	-	-	0.55	-	-	-
Protein g	40.0	6.0	6.0	-	20	-	10.3	-
Fat g	8.6	7.6	7.6	-	13.3	-	0.2	-
Carbohydrate CHO g	55.0	57.0	57.0	-	50	0 later10	15.3	13.3
Calories kcal	470	322	322	-	400	3 later 43	119	59

1.5.2. Micronutrient supplementation during pregnancy

Interest has recently increased in the possible role of multiple-micronutrient deficiency as a growth limiting factor during pregnancy and contributing to the metabolism and development of the foetus in utero and the infant postnatally. It has been hypothesized that micronutrient deficiency in undernourished pregnant women changes the maternal haemo-dynamics which interferes with feto-placental transportation of nutrients (Rosso et al, 1994). Moreover, many micronutrients are integral components of body tissues and some play an important role in metabolism, gene transcription, immunity, endocrine regulation and nutrient transportation (Villar et al, 2003; Keen et al, 2003).

It is also questioned whether prenatal micronutrient supplementation alters foetal brain development and contributes to subsequent motor, cognitive and behavioural development in addition to improved physical growth. However very little information is available about multiple-micronutrient supplementation during pregnancy at the period when brain growth spurt occurs and when its consequences on subsequent child development may be greatest.

Governments and international agencies, particularly in developing countries, are anxious to reduce the intergenerational-malnutrition cycle (mother-child-mother) and its consequences, but there is insufficient information to guide policy. From a policy point of view, supplementing pregnant mothers with multiple micronutrient tablets was considered to be a more feasible approach than providing micronutrient-balanced food. Through multiple micronutrient supplementations it is possible to provide several key nutrients like iron, folic acid, vitamin B complex, antioxidants etc in a single tablet in a cost effective way.

There remain a number of problems in formulating an efficient mixture of multiple micronutrients to correct the deficiencies in nutritionally vulnerable populations. *Firstly*, it is difficult to assess the actual requirement of certain micronutrients like zinc, due to the absence of a sensitive biochemical determinant (Whittaker, 1998). *Secondly*, there is evidence that metabolic interaction occurs among different micronutrients; however the mechanism is not always clearly understood.

Some micronutrients compete for the same binding site for absorption at different concentrations. Non haem iron can inhibit the absorption of zinc (Solomons & Jacob, 1981; Valberg et al, 1984; Sandstrom et al, 1985) and vice versa (Crofton et al, 1989; Rossander-Hulten, 1991) probably by competing for the absorption sites in the small intestine. In animal models an iron transporter in the apical side of enterocytes has been discovered which can also transport zinc ions (Eisenstein et al, 1998; Gunshin et al, 1997). For this reason Lind and colleagues (2003) found iron-zinc combinations in oral-supplementation to be less efficacious in improving the blood levels of both mineral than supplementing with either one alone. Copper absorption is also hampered by high zinc intake in experimental animals (Wapnir et al, 1993). In human subjects high intake of zinc interferes with absorption of iron and copper, eg in Wilson's disease intake of zinc >50 mg/day is recommended to reduce copper load (Marcellini et al, 2005; Ferenci, 1998).

In contrast, some micronutrients facilitate the absorption of others. Ascorbic acid enhances absorption of non haem iron (Teucher et al, 2004). Vitamin A facilitates the utilization of ingested iron, transport of iron and production of haemoglobin, thus improving iron stores (Suharno et al, 1993; Mejia LA, 1988, Lynch, 1997). Zinc plays role in the conversion of beta-carotene to retinol (Dijkhuizen et al, 2004). Zinc also increases plasma retinol binding protein and mobilization of vitamin A (Christian & West, 1998; Baly et al, 1984). Thus zinc deficiency can aggravate hypovitaminosis A as it is required for transport of hepatic vitamin A to target tissues. Selenium is a key component of the enzyme which is required to convert thyroxine to tri-iodo thyronine, so its deficiency can impair utilization of iodine by tissues. Riboflavin has an important role in absorption, metabolism and utilization of iron (Powers, 2003). Vitamin E protects vitamin A and ascorbic acid from oxidation. Magnesium facilitates absorption of calcium from the gut. Similarly, calcium helps in absorption of vitamin B12 from the ileum but it interferes with absorption of zinc (Singh, 2004). *Thirdly*, it is difficult to determine in which form a supplementation of micronutrient-mix will exert maximum effect. For example, an interaction of iron and zinc is reported to be evident when they are given in solutions but not when served with meals (Sandstrom et al, 1985; Rossander-Hulten et al, 1991; Davidsson et al, 1996; Haschke et al, 1986; Fairweather-Tait et al, 1995). In contrast, some studies have reported that fortification of infant formula with high iron can interfere with

absorption of copper but not when given alone as a supplement (Lonnerdal et al, 1994; Haschke et al, 1986). For example, food based zinc supplementation of very malnourished infants with persistent diarrhoea resulted in lower concentrations of plasma copper level (Bhutta et al, 1999), but did not interfere with the serum copper status of infants when given in a supplemented form (Lind et al, 2003). *Fourthly*, sometimes side effects like vomiting reduce the compliance of multiple micronutrient-mixes and make it difficult to assess the exact role in modifying biochemical levels. For example, there are reports of increased vomiting in infants taking combined micronutrients (Black et al, 2004a; Lind et al, 2003) which might hinder the optimal benefit of a combined supplementation. *Finally*, host factors, like individual micronutrient status, presence or absence of inflammation (Tomkins, 2003), dietary habits, age etc of the host at the time of supplementation, greatly modify the effect of individual or group of nutrients.

In this section I will review selected literature concerning the effect of micronutrient supplementation during pregnancy on birth outcome and child development. The topics will be discussed under following sub-headings:

- Intrauterine micronutrient supplementation - effect on birth measures
- Intrauterine micronutrient supplementation - effect on child development

(i) Intrauterine micronutrient supplementation, effect on birth measures

Here I discuss only the randomized controlled trials (RCTs) with >10 multiple micronutrients (vitamin and minerals) during pregnancy that looked for their effect on birth outcome. Following this I briefly discuss studies of supplementation in pregnancy with fewer micronutrients.

Search criteria: To locate the RCTs I carried out a MEDLINE search using PubMed. The search was restricted to all randomized control trials done before 2005 on human-subjects only. From there I selected only those studies that supplemented apparently healthy pregnant mothers at any time after conception.

Trials which targeted pregnant mothers with any known illness like HIV, malaria or diabetes were excluded because the mechanism of action of micronutrients can be

very different in these mothers and can be difficult to generalize. The chosen phrases for this search were – “pregnancy” and/or “micronutrient”, “vitamins”, “minerals”, “individual name of micronutrients”, “birth weight”, “preterm birth” and “low birth-weight”.

Table 1.15. presents all the prenatal-RCTs with multiple micronutrients on apparently healthy mothers that measured the effect on birth outcomes. I shall also report a summary of some relevant review articles and discuss RCTs with individual micronutrients that looked at birth measurements. To make the tables simple, I shall not present any maternal or foetal haematological and biochemical profiles. Information on important biochemical changes in these studies will be discussed in the text if necessary.

Multiple- micronutrients (>10 micronutrients)

I could locate only 5 recent randomized control trials that looked for effect of multiple-micronutrient supplementation during pregnancy on birth measures (Table-1.15). The composition of multiple micronutrients used in these studies is presented in Table-1.16. All the studies used >13 micronutrient and vitamin combinations. Two of them were conducted in Nepal, one in Mexico, one in France and one in Zimbabwe, thus four were from developing countries and one from a developed country. All the studies, except the French one (Hininger et al, 2004), were epidemiological studies with large sample sizes. Both the Nepalese (Osrin et al, 2004; Christian et al, 2003) studies showed positive impact of prenatal multi-micronutrient supplementation. The most recent one (Osrin et al, 2004) was a well designed study with minimum loss. However they were recruited from clinics to form a mixed population from different social backgrounds, though the measured socio-economic variables and participants’ characteristics were similar in both the groups. It is possible that the population who did not attend the clinics (the very poor or the more affluent) might vary in their response to supplements and there are concerns about the external validity of the study. But, overall, the fall in proportion of low birth weight of 25% is impressive.

The other Nepalese study (Christian et al, 2003) that supplemented micronutrients in 5 different combinations, also showed benefit from prenatal supplementation with 14

multiple micronutrients (significant 64 g increment in birth weight and 14% reduction of LBW) and from routine iron-folate combination (non-significant 37 g increment and 16% reduction of LBW) (Table 3.15.). Among the groups receiving different types of supplements, only the iron-folate and multiple-micronutrient groups showed a positive impact on birth weight. However when comparing the multiple-micronutrient group with the iron folate group, which is comparable to the comparison made in the study by Osrin and colleagues (2004), the multiple-micronutrient group was only 27 gram heavier and the difference was not significant. Interestingly, benefits from iron-folate supplementation were no longer seen in the group where zinc was added to them (iron-folate-zinc group). But zinc was present in the multiple-micronutrient group and the benefits on birth weight were still apparent. Here the authors speculated that by including zinc in the mixtures the observed variation might be due to competition for absorption with iron or interactions among the other micronutrients (Solomons, 1986; 1997; Whittaker, 1998). The study also reported that increments in birth weight in the multiple-micronutrient group were disproportionately greater at the upper end and over 50% more infants had birth weight ≥ 3300 in the multiple-micronutrient group than the control group. However, in the iron-folate group, the distribution of birth weight was the same as the controls. Finally the author concluded that unlike the other Nepalese study (Osrin et al, 2004) there was no extra benefit of multiple micronutrients over iron-folate mix, which was the routine practice for pregnant mothers.

Among the other three randomised control trials, one showed significant benefits (Hininger et al, 2004) on birth outcomes, one showed possible benefits (Friis et al, 2004) and one showed no benefits (Ramakrisnan et al, 2003). The French study that showed benefits (Hininger et al, 2004) had a very small sample size, calculated mainly on the basis of improvement in biochemical parameters. It was too small to draw meaningful conclusions on birth measures; also the study had a big loss of subjects but reported nothing about this. The study also found higher maternal blood vitamin concentrations for vitamins C, E, B₂, B₆ and β -carotene in the supplemented group. They also found a lower number of infants with birth weight < 2700 g in the supplemented group and birth weight below this cut-off has been reported to have increased risk for morbidity (McIntire et al, 1999). In the study in Zimbabwe (Friis et al, 2004) there was a tendency for multiple micronutrients to benefit birth weight but

the improvement was not statistically significant ($p=0.08$). However the population of this study was mixed with asymptomatic HIV infected mothers (diagnosed after randomization) and there was a large loss of 34% of subjects. In addition, as an effectiveness trial, all calculated supplements were given to the mothers at the same time and compliance was not monitored, thus it is unknown how much supplementation was taken. Finally, in the Mexican study (Ramakrisnan et al, 2003) the researchers found no benefit of multiple micronutrients, although the study had a good compliance. Perhaps deficiencies of multiple micronutrients were not big enough to affect foetal growth.

Single or <10 micronutrients (not shown in tables)

Supplementation with single micronutrients is not always beneficial and might even be detrimental, possibly due to adverse or inhibitory effects on absorption of other micronutrients. Not all the individual micronutrients in a multiple-micronutrient tablet have been observed for their effects on human pregnancies.

Out of the 15 micronutrients in the UNICEF/WHO/UNU multiple-micronutrient tablet used in my study, only 6 micronutrients (zinc, iron, folic acid, vitamin D, vitamin A and iodine), individually or in combination, have been used in pregnancy trials (Ramakrisnan et al, 1999). I shall discuss the trials and reviews of single or <10 micronutrient mixes in the following text.

Zinc (RCTs) - A Cochrane systematic review on 5 methodologically sound zinc trials during pregnancy failed to find any beneficial effect on LBW or other growth measures (Mahomed, 2000a). In a review of 13 zinc-supplementation trials during pregnancy, Shah and Sachdev (2001) could not find any consistent benefit on birth outcomes. In that review all but three studies were from a developed country and possibly did not address a zinc deficient population. Out of these three studies, two - one from South Africa (Ross et al, 1985b) and one from Bangladesh (Osendarp et al, 2000) - found no benefit with supplementation on birth outcome. But the third study from India (Garg et al, 1993) showed a significant increment of birth weight (300-800 gram) in the zinc-supplemented group. However these three studies varied largely in the dose of zinc supplementation - the South African, Indian and Bangladeshi studies used <12.9 mg, 45 mg and 30 mg zinc/day respectively. The

sample size of the South African study was very small (supplemented/control=32/36) which might cause type-II error in interpreting birth-effect (Ramakrishnan et al, 1999). Moreover, the groups were not comparable (greater number of lighter mothers in the supplemented than the control group). In one recent RCT in Pakistan (Hafeez et al, 2005), pregnant women were supplemented with zinc (20mg/day, n=121) or placebo (glucose, n=121) from 10-16 weeks gestation until delivery. The results failed to show any benefit of zinc supplementation on birth weight. However this study also had methodological limitations like selection bias (recruited hospital delivery cases only) and inadequate sample size.

Iron and iron- folate RCTs : Iron supplement trials in India, Gambia and Nigeria did not demonstrate significant benefit on birth weight (Sood et al, 1975; Fleming et al, 1986; Menendez et al, 1994; Preziosi et al, 1997). Cochrane systematic reviews done on 20 trials with iron (Mahomed, 2000b) and 8 trials with iron with folic acid (Mahomed, 2000c), showed that iron alone, or combined with folate, improved the hematological profile in pregnant mothers. However their role on pregnancy outcome was not clear and few data were derived from communities where iron deficiency or anaemia was prevalent. In addition most of the studies compared different doses or route of iron administration in pregnant women due to ethical concerns about withholding iron when there were global recommendations to give iron and folate to pregnant mothers.

One recent study (Cogswell et al, 2003) in a low-income iron-replete population of Cleveland, compared iron supplemented (30mg/day, n=146) pregnant mothers with a non-supplemented group (placebo, n=129). Supplementation started <20weeks of gestation until delivery. The iron supplemented group had significantly higher birth weights (206 g heavier; P=0.010) and lower incidence of low-birth-weight infants (4% compared with 17%; P=0.003) than placebo group. These results also need to be interpreted with caution due to the methodological limitations. Firstly, the placebo group also received iron supplementation after 28 weeks of gestation because most (78%) of them developed depleted or absent iron stores at that stage. Ultimately all mothers of the placebo group received iron supplementation for the rest of their pregnancy. So the comparison was actually early versus late supplementation. Secondly, the sample was too small to observe the effects on birth outcomes with

confidence. According to Ramakrishnan and colleagues (1999), a minimum sample of 200 is required in each group in supplementation studies to detect a biologically meaningful 100 g difference between groups.

Vitamin A - Only one recent study from Indonesia (Schmidt et al, 2002) could be located that looked at the effect of weekly vitamin A (4800 RE) supplementation during pregnancy on birth weight. In addition, both the supplementation and control groups received equal amounts of weekly iron (120 mg) and folate (500µg) from the ~18th week of gestation until birth. The study found no effect of adding vitamin A supplementation on birth weight or subsequent growth. Though the dose of vitamin A was small it was within the WHO recommended dose for pregnant women and was safe. The author questioned whether giving iron and folate to the placebo group concealed the effect of vitamin A (if any) on birth weight and growth. However no firm conclusion can be drawn about the role of vitamin A during pregnancy on birth weight from only one RCT.

Vitamin D, B6, and iodine - In a recent review of pregnancy-trials with micronutrients, Fall and colleagues (2003) reported only 2 RCTs with vitamin D that came up with conflicting results. One showed a birth weight increment while the other showed no such improvement. The review also cited one supplementation trial in Taiwan with vitamin B6 (Chang, 1999) and one in Algeria with iodine (Chaouki et al, 1994), both trials showed significant increments of birth weight. I could locate no new RCTs with these micronutrients from a recent search. However it is difficult to draw definitive conclusions from such a small number of studies where most of them had methodological problems regarding power of the study, dose of supplements and controlling confounders.

Summary on RCTs of multiple micronutrients during pregnancy on birth-outcome of offspring:

I identified 5 published RCTs on the effect of multiple micronutrients given to pregnant women on their infants' birth weight. Four of them (Osrin et al, 2004; Christian et al, 2003, Friis et al, 2004; Ramakrishnan et al, 2003) had a substantial number of subjects and were from developing countries, whereas one small one was from France (Hininger et al, 2004).

All the studies compared multiple micronutrients (> 10 micronutrients and vitamins) with one or more comparison groups, three of them had a group receiving iron-folate supplement for comparison (Osrin et al, 2004; Christian et al, 2003, Friis et al, 2004), one used only iron (Ramakrishnan et al, 2003), and one used a placebo (Hininger et al, 2004) but the composition was not mentioned (Tables 1.15. & 1.16.). Study results varied from no significant (Ramakrishnan et al, 2003) benefit to possible benefit (Friis et al, 2004), to clear benefit (Osrin et al, 2004; Christian et al, 2003; Hininger et al, 2004).

However, the study by Christian and colleagues (2003) only showed a significant benefit when the multiple-micronutrient group was compared with a group receiving only vitamin A supplementation, whereas the benefit compared with iron and folate was not significant. In general all but one study (Ramakrishnan et al, 2003) with multiple micronutrients during pregnancy showed a consistent improvement in birth weight although not always significant.

The difference in birth weights between the multiple-micronutrient and iron-folate groups for three studies was 77g (Osrin et al, 2004), 49 g (Friis et al, 2004) and 27 g (Christian et al, 2003). In the other two studies the micronutrient group showed an improvement of 251 g (Hininger et al, 2004) compared to placebo and 4 g (Ramakrishnan et al, 2003), compared to the iron group. The one published study (Ramakrishnan et al, 2003) that found no benefit with micronutrients did not address a population at risk - a third of the mothers were over-weight and the rate of LBW was less than 9%. This population was also not deficient enough in micronutrients to benefit from supplementation.

In addition preliminary results on birth-outcome are available from three large RCTs (unpublished preliminary report, personal communication: Report for MINIMat team only, Lars Ake Persson) including the one in Bangladesh that is the subject of this thesis. The other two studies were done in Pakistan and Guinea Bissau. All three studies found very small and non-significant improvements in birth weight (16g, 53 g and 40 g in Bangladesh, Pakistan and Guinea Bissau). Considering the results of birth weight from the above 8 studies there is a consistent but very small improvement in the three studies (Osrin et al, 2004; Christian et al, 2003; Hininger et

al, 2004), but the difference is unlikely to have functional significance or public health importance.

None of the trials reported any harmful effects from the supplementation. However the two Nepalese studies, in a recent letter to the Lancet, reported a significantly higher peri-natal mortality rate when the studies were combined in the infants where mothers received multiple-micronutrients supplementation (Christian et al, 2005).

A trial was carried out in Tanzania (Fawzi et al, 1998) but is not included in this review as it targeted asymptomatic HIV infected pregnant mothers. Mothers were supplemented with high doses (2-20 times RDA) of multiple-micronutrients and showed a great fall (44%) in LBW and preterm delivery (39%). This study suggests that the role of micronutrients in pregnancy complicated with HIV may be different and looks promising.

One problem in generalizing the role of micronutrient-mixes is that different studies used different combinations of micronutrients with varying doses and targeted different types of populations. For example, the two Nepalese studies which addressed rural (Christian et al, 2003) and urban (Osrin et al, 2004) populations of Southern Nepal, used 15 multi-micronutrient-mixes with slightly varying formulations. One had iodine and selenium with 30 mg iron and 15 mg zinc in the mix and showed a significant increase in birth weight over the iron-folate group (Osrin et al, 2004). The other one had no iodine or selenium but manganese with 60 mg iron and 30 mg zinc and failed to find significant increments in birth weight over the iron-folate group. Most of the studies tried to remain close to one RDA.

Interaction between the micronutrients and biochemical mechanisms further complicates the situation. Some studies omitted potentially beneficial micronutrients to avoid possible interactions but eventually found other micronutrients in the composition to be interactive. For example, the French study by Hininger et al (2004) excluded iron from the multi-micronutrient-mixes because of its oxidative potential (Halliwell & Gutteridge, 1990) but finally assumed that folic acid competed with zinc to result in low plasma zinc levels.

Similarly, to explain not getting the expected benefit from prenatal micronutrient supplementation, even after having a very good compliance, Ramakrishnan et al (2003) hypothesized that a possible interactive role of zinc hampered other potential micronutrients from exerting their effect (Caulfield et al, 1999; Osendarp et al, 2000). Trials with individual micronutrients are helpful to understand their individual mechanism of action; however they may behave differently when combined with other micronutrients.

Therefore we can conclude that the review noted some evidence of benefit on birth weight from supplementing with multiple micronutrient-mixes during pregnancy. However, when compared with giving iron-folate, the benefits were either non significant or were usually so small as to be unlikely to have functional significance. Furthermore, the recent report of increased perinatal mortality needs careful consideration and further investigation of the existing data sets.

Table 1.15. Effects of multiple-micronutrient supplementation during pregnancy on BW (Randomized control trials)

Study	Design and supplementation	Sample	Measures	Results	Comment
Nepal Osrin et al, 2005.	Double-blind, RCT multiple-micronutrient group*: UNICEF/WHO/UNU 15 Multiple micronutrient (single RDA) Control: Iron 60mg + folate 400µg Dosage: 1 tab daily after or with food ~ 12 weeks of gestation until delivery	Pregnant mothers < 20 weeks of gestation Multiple-micronutrient*group: n=600 Control: n=600	Birth weight Gestational age Birth length Head circum-ference	Between two groups – Birth weight: Significant 77 g increase (95% CI 24-130; p=0.004) in multiple-micronutrient group than control group Low birth weight: 25% reduced in multiple-micronutrient group than control group Gestational age: Not significant, 1-5 days prolonged in multiple-micronutrient group than control group Infant length : Not significant Difference Head circumference: Not significant Difference	Positive finding Well designed study Only 12-13% loss of subjects. Selection bias in sampling, reported on mothers who came for regular antenatal visit. The population was urban-rural mixed population & moderately poor
Zimbabwe Harare. Friis et al, 2004	Randomized placebo-controlled, double-blind effectiveness trial multiple-micronutrient* group: 13 micronutrients and iron (30 mg) + folate (0.4 mg) part of antenatal care and iodine by iodized salt. Control: Iron + Folate Dosage: 1 tab daily during pregnancy until delivery. All calculated tablet was given once, not monitored.	1669 pregnant women (22-36 wk of gestation) who attended antenatal care with or without HIV infection. Multiple-micronutrient*group: n=837 Control: n=832	Birth weight Gestational age Birth length Head circum-ference	Between two groups – Birth weight : 49 g higher (CI: -6, 104;P= 0.08) in multiple-micronutrient group than control group. Gestational age: 0.3 week higher (95% CI: - 0.04, 0.6; P = 0.06) in multiple-micronutrient group than control group Head circumference: 0.2 cm higher (CI: -0.02, 0.4 ; P = 0.07) in multiple-micronutrient group than control group Low birth weight (%): Not significant difference between the groups	Tendencies towards positive finding Big loss 34% compromising the statistical power. Adherence to supplementation was not optimal. The population was mixed, a third of tested mothers having asymptomatic HIV infection

* Composition of multiple micronutrient mixture was given in Table 1.16.

CI= Confidence interval; n=number of subjects, MN= Multiple micronutrient; RCT= Randomized control trial; RDA=Recommended daily allowance; Zn= Zinc;

Table 1.15. (continued) Effects of multiple-micronutrient supplementation during pregnancy on BW (Randomized control trials)

Study	Design and Supplementation	Sample	Measures	Results	Comment
France Hininger et al. 2004.	Double-blind, randomized controlled intervention trial. multiple-micronutrient* group: Iron-free 12 micronutrients Placebo- Composition not mentioned Dosage: 1 tablet daily until delivery.	100 apparently healthy pregnant women enrolled at 14+/-2 weeks. Total 65 women completed the study. Multiple-micronutrient* group: n=33 Placebo: n=32	Birth weight Gestational age Birth length Head circumference	Between two groups – Birth weight: Significant 251 gram (10%) higher (P=0.03) in multiple-micronutrient group than placebo group Birth length : Difference not significant Head circumference: Difference not significant Birth weight : <2700 g =Significantly lower (P=0.02) in multiple-micronutrient group than placebo group Gestational age: Difference not significant	Positive finding Sample size was inadequate for birth outcome. Loss was big, 35% but nothing reported about it. The study was designed for biochemical parameters.
Mexico Ramakrishnan et al., 2003	Randomized controlled community trial multiple-micronutrient * group: 13 Micronutrient (1-1.5 times the RDA) and 60 mg iron Control: 60 mg iron Dosage: 1 tab daily for 6 days/week at home, as well as routine antenatal care, until delivery.	Pregnant women (n = 873) at < 13 week of gestation Multiple-micronutrient*group: n = 323 Control group: n = 322	Birth weight Birth length Head circumference Gestational age	Between two groups Birth weight: Difference not significant, only 4 gram higher in multiple-micronutrient group than control group Birth length: Difference not significant Head circumference: Difference not significant Duration of gestation: Difference not significant	No benefit Compliance 95% Big loss 26% but sample size remained adequate Population was from semi rural area with low prevalence of anemia (10-14%) and low birth weight (<10%)

* Composition of multiple micronutrient mixture was given in Table 1.16.

CI= Confidence interval; n=number of subjects, MN= Multiple micronutrient; RCT= Randomized control trial; RDA=Recommended daily allowance

Table 1.15. (continued) Effects of multiple-micronutrient supplementation during pregnancy on BW (Randomized control trials)

Study	Design and Supplementation	Sample	Measures	Results	Comment
Nepal Christian et al, 2003	<p>Double blind randomized controlled community trial 426 communities were randomized to five regimens</p> <p>Group (FA): Folate + vitamin A</p> <p>Group (FA+Fe): Folate +iron + vitamin A</p> <p>Group (FA+Fe+Zn): Folate + iron+ zinc + vit A</p> <p>Group (MN): 14 multiple micro nutrients* + vitamin A</p> <p>Group (control): Vitamin A (1000 µg)</p> <p>Dosage: 1 caplet daily at bed time until delivery, monitored twice weekly</p>	<p>4926 pregnant women and 4130 live born infants.</p> <p>Group (FA): n=628</p> <p>Group (FA+Fe): n=635</p> <p>Group (FA+Fe+Zn): n=672</p> <p>Group (MN): n=705</p> <p>Group (control): n=685</p>	<p>Birth weight</p> <p>Birth length</p> <p>Head circumference</p> <p>Chest circumference</p> <p>Gestational age</p>	<p>In different groups compared to control-</p> <p>Birth weight: Group (FA)= Difference not significant Group (FA+Fe)= 37 g higher (CI: -16, 90) Group (FA+Fe+Zn)= Difference not significant Group(MN)= 64 g higher (CI: 12 , 115)</p> <p>Birth length : Difference not significant</p> <p>Head circumference: Group (FA+Fe)=0.16 cm higher (CI: -0.03, 0.34) Group(MN)= 0.19 cm higher (CI: 0.02 to 0.37)</p> <p>Chest circumference Group (FA+Fe)= 0.35 cm higher (CI: 0.09, 0.61) Group(MN)= 0.36 cm higher (CI=0.11 to 0.61)</p> <p>Low birth weight Group (FA+Fe)= 16% reduced (RR= 0.84, 0.72 to 0.99) Group (MN)=14% reduced (RR=0.86; 0.74 to 0.99)</p> <p>Preterm birth (<37 weeks)- Difference not significant</p>	<p>Positive finding Higher birth weight Adequately powered study. Field performance & compliance (median 88%) are good. Findings suggests no added benefit of multiple micro-nutrient over iron+ folate supplement</p>

* Composition of multiple micronutrient mixture was given in Table 1.16.

CI= Confidence interval; Fe= iron; FA= folic acid, n=number of subjects, NS= not significant difference, MN= Multiple micronutrient; RCT= Randomized control trial;

RDA=Recommended daily allowance; Zn= Zinc

Table 1.16. Composition of different multiple micronutrients used in 5 randomized control trials in Table 1.15

MN	Nepal Study Osrin et al, 2005	Zimbabwe Friis et al, 2004	Nepal Christian et al, 2003	Mexico Ramakrishnan et al, 2003	France Hininger et al, 2004
	15 micronutrients	13 micronutrients	14 micronutrients	13 micronutrients	12 micronutrients
Vit A µg	800 µg	3000 µg	1000 µg	2150 IU	--
– β-carotene mg		3.5			4.8
Vit D	5 µg	10 µg	10 µg	309 IU	--
Vit C Ascorbic acid mg	70	80	100	66.5	60
Vit K µg			65		--
Thiamine B1 mg	1.4	1.5	1.6	0.93	1.4
Riboflavin (B2) mg	1.4	1.6	1.8	1.87	1.6
Niacin mg	18	17	20	15.5	15
Pyridoxine (B6) mg	1.9	2.2	2.2	1.94	--
Cyanocobalamin (B12) µg	2.6	4	2.6	2.04	--
Cobalamin µg					1
Vit E mg	10	10	10	10	10
Pantothenic acid mg					6
Calcium (Ca) mg					100
Magnesium (Mg) mg			100	252	87.5
Iron (Fe) mg	30	30	60	62.4	--
Folic Acid (FA) µg	400	400	400	215	200
Zinc (Zn) mg	15	15	30	12.9	15
Selenium (Se) µg	65	65			
Iodine (I) µg	150				
Copper (Cu) mg	2	1.2	2		

(ii) Intrauterine micronutrient supplementation, effect on child development

There are very few data on the effects of micronutrients during pregnancy on an infant's cognitive development. As with lack of energy and protein, micronutrient deficits tend to be associated with poverty which independently affects child development. It is therefore necessary to have randomized trials to establish causal relationships.

In human subjects, pregnant women with moderate to severe iron, zinc and folic acid deficiencies reported to suffer from an increased risk of low birth weight, pregnancy complications and birth defects (Seshadri et al, 2001). So far only zinc, iron, folic acid and vitamin A supplementation during pregnancy have been observed for an effect on later child development, either alone or in combination. Though the role of prenatal iron-supplementation on subsequent child development in nutritionally deficient populations is not very clear, its effect on prenatal brain development in animal models is well documented (Kwik-Urbe, 2000). Animal studies have shown that chronic marginal Fe intakes during early development result in persistent biochemical and behavioural changes that were not reversed by postnatal supplementation (Kwik-Urbe, 2000). Kilbride showed in her study that infants born to anaemic mothers were mostly anaemic (Kilbride et al, 1999) thus very likely to suffer from poor cognitive development and behaviour (Black, 2003; Angulo-Kinzler, 2002). Marginal or severe vitamin B6 restriction in animals during gestation also showed reduction of neuronal connection and thus on the functional consequences of the offspring (Kirksey et al, 1990).

In this section I shall describe pregnancy-studies with single multiple micronutrients looking for their effect on subsequent child development.

Search criteria: For this purpose I carried out a MEDLINE search using PubMed by choosing the phrases - 'pregnancy', 'micronutrient', 'individual name of vitamin and minerals', 'child development', 'cognition', 'motor development', 'intelligence', 'behaviour' and 'activity'. My search remained restricted to all micronutrient studies during pregnancy on human subjects. By using the term "child development" I mainly focused on cognitive development, motor development and behaviour.

However as some of the congenital neuronal developmental (neural tube closure) issues came as a part of pregnancy-micronutrient-studies, I described them along with studies of child development.

In the following section I discuss iodine and folic acid as they have a well-established beneficial role in neural or child development. Only the micronutrients that still have a controversial role in child development are presented in the tables.

This will be followed by a discussion of micronutrient supplementation during the “periconceptional period”, studying their role on neural or developmental measures of the offspring. Finally I shall present studies with micronutrients during pregnancy that assessed mainly child development.

Micronutrients with established role on neural development or child development during pregnancy

Iodine - So far the role of iodine during early pregnancy on foetal brain development is reasonably well established (Grantham-McGregor & Ani, 1999). In Papua New Guinea endemic cretinism was eliminated by preconceptional injections of iodized oil in a double blind RCT (Pharoah et al, 1971). This study clearly established the causal role of iodine deficiency in the multiple neurological deficits in cretinism. The authors also reported better cognitive and motor functions among 10 year old non-cretin children whose mothers received preconceptional iodized oil treatment (Pharoah & Connolly, 1987). Consistent beneficial effects of iodine treatment given before conception (Pretell et al, 1972) and in early pregnancy (Thilly et al, 1973) on the development of the offspring have also been observed in other studies.

Folic acid - One multi-centre trial with folic acid along with other multiple-micronutrient supplementation of pregnant mothers with a history of previous neural tube defects (NTD), showed 60% reduction in NTD (Central Technical Co-ordinating Unit, 2000), but this difference was not significant. In a much larger randomized double-blind prevention trial with a factorial design, folic acid reduced neural tube defects by 72% compared to a mixture of 7 vitamins in mothers with a history of a previously affected pregnancy (MRC Vitamin Study Research Group, 1991). This study established the benefits of folic acid in a high risk population. Thus, the role of folic acid deficiency on neural development became well

established (Fernandez-Ballart & Murphy 2001). However folic acid was also found to have roles other than neural tube maturation. A review on folic acid (folate) that looked at both observational and supplemental studies suggested that poor dietary folate intake and low circulating concentrations are associated with increased risk of adverse birth outcomes including abortion, growth restriction and with neural tube defects (Scholl & Johnson 2000).

The effect of periconceptional micronutrient-mix supplementations on neuronal or child development

Table 1.17. presents only one RCT of supplementing pregnant women with multivitamins including folic acid for 2 months around conception and showed no effect on the rate and distribution of ophthalmologic and audiological disorders, growth and mental development of the offspring at 2 and 6 years of age (Dobo & Czeizel 1997, 1998). It did find significantly lower anxiety levels in children of the supplemented group.

Table 1.17. presents 3 other studies that looked for an association of periconceptional diets and multivitamins on neuro-developmental outcomes. Two of them (Botto et al, 2002; Groenen et al, 2004) were case-control studies and compared mothers who had children with non syndromic meningo (myelo) cele with mothers who had normal healthy children. The results showed a lower risk of neural tube defects in children of mothers who took periconceptional micronutrients. However the findings were biased with maternal recalls. Dietary recall of the mothers showed that low pre-conceptional intakes of plant proteins, Fe, Mg, and niacin are associated with a 2- to 5-fold increased risk of spina-bifida (Groenen et al, 2004). The other population based case control study in the USA (Botto et al, 2002) looked retrospectively at cases of non syndromic omphalocele and normal healthy infants and compared exposure of their mothers to multivitamins during the periconceptional period. Results show a 60% reduction in the risk for nonsyndromic omphalocele infants in mothers who used periconceptional multivitamins. However the composition of micronutrients was not mentioned and it seems that maternal recall was collected for any type of commercially available multiple-micronutrient mixes.

The third study (Holmes-Siedle et al 1992) was a prospective long term follow-up study, where all women with a previous history of one or more pregnancies complicated by a neural tube defect were supplemented with periconceptional multivitamins. Supplemented mothers showed significant reduction in recurrence of birth defects. But long term follow-up showed more frequent worries and fearfulness among children of supplemented mothers at the age of 7-10 years. However there was no control group and the findings were compared with the general population norms.

Studies during pregnancy with single and ≥ 2 micronutrients on child development

Tables 1.18. and 1.19. presents details of some studies that looked at the association of different micronutrients during pregnancy with child development. I will describe studies with a combination of two or three micronutrients first then studies with single ones. In the table observational studies will be described first and will be followed by stronger case control studies and then RCTs.

Studies with ≥ 2 micronutrients - Only 2 studies (Table 1.18.) were identified that looked at the effect of two or more micronutrients through-out pregnancy (not with energy or protein) to see their effect on subsequent child development. In one RCT, Meraldi and colleagues (1999) assessed foetal neurobehavioural development (heart rate and movement pattern) on 55 fetuses using electronic monitors at ~36th week of gestation whose mothers were supplemented with daily iron-folate with or without 15 mg zinc. The result showed a beneficial effect on improved heart rate and activity of fetuses of zinc-supplemented mothers. However this result should be interpreted with caution due to its small sample size and methodological limitations, eg like validity of tests. Another recent RCT from Indonesia (Schmidt et al, 2004) looked at the effect of a weekly iron-folate supplementation of pregnant mothers with or without vitamin A and compared daily iron-folate supplementation, routinely offered by government health services, on subsequent mental and motor development of their infants. The infants were assessed at the age of 6 and 12 months using the Bayley scales and failed to show any benefit from vitamin A or any difference between weekly or daily iron-folate supplement.

Studies with single micronutrients - Among the single-micronutrient supplementation studies, I could locate one observational study and two RCTs that focused on the effect of zinc alone and only one that focused on iron alone during pregnancy on child development (Table 1.19.).

Zinc- An observational study from Egypt reported that infants of mothers who took diets containing high levels of animal food, which is rich in zinc, were more attentive soon after birth than infants of mothers who had low zinc intakes (Kirksey et al, 1991). Subsequently the follow up of this study documented the persistence of this positive association with motor development at the age of 6 months (assessed using Bayley Scale of Infant Development) (Kirksey et al, 1994). Of the two RCTs, one in Birmingham, USA found no effect of supplementation during pregnancy on child development at 5 years of age (Tamura et al, 2003). In this study the population were African-American women from low socio-economic status, apparently with inadequate zinc. However, previously these infants had benefited from prenatal zinc supplements in their birth measures (increased birth weight and head circumference), in the subgroup of mothers with BMI<26 kg/m² (Goldenberg et al, 1995). This finding was important as it suggests that there is no long-term developmental consequence of infants who had increased head size at birth from prenatal zinc supplementation. However, as the study was done in a developed country, it is difficult to generalise the finding. On the other hand, the Bangladesh study, with zinc supplementation during pregnancy, found significantly lower developmental scores among children of supplemented mothers (Hamadani et al, 2002), but the deficit was small which may not have functional significance and the study suffered considerable loss.

Iron - I could locate only one RCT (Zhou et al, 2004) that looked at the effect of iron supplementation alone during pregnancy on the development of infants at the age of 4 years. The study found no benefit on the intelligence quotient (IQ) of children of supplemented mothers when tested by Stanford Binet Scale.

Summary of micronutrient supplementation studies during pregnancy on child development: Other than studies on the established role of iodine and folic acid during pregnancy, I could locate only 10 studies that looked for an association of

different doses and combinations of micronutrients around pregnancy with subsequent neuronal or child development of the offspring.

Four studies concentrated the use of micronutrients during the peri-conceptional period and its association with infants' neural tube defects and behaviour. All were from developed countries (Dobo & Czeizel 1997, 1998; Groenen et al, 2004; Botto et al, 2002; Holmes-Siedle et al 1992). Two used multiple-micronutrient supplementation, one was an RCT and the other supplemented mothers who had a previous child with a neural tube defect and compared them with a normal population. Both the studies found incidence of reduced neural tube defects but no effect on IQ or educational attainment. One found reduced anxiety (Dobo & Czeizel 1997, 1998) and the other found increased anxiety (Holmes-Siedle et al 1992). The other two periconceptional case-control studies also showed an association between reductions in neural tube defects and early micronutrients containing folic acid (Botto et al, 2002), or a combination of iron, magnesium and niacin (Groenen et al, 2004). These studies did not look for any other developmental and behavioural aspects of infants. Therefore in the periconceptional period, multiple micronutrients showed a consistent beneficial association with reduced neural tube defects, but there was no effect on later development and an inconsistent finding on the behavioural outcomes. In addition, all the studies had design problems. For example, the RCT (Dobo & Czeizel, 1997, 1998) had no proper placebo group and the comparison of multiple micronutrients with trace elements made the interpretation difficult. Similarly, the prospective study (Holmes-Siedle et al, 1992) had no control group at all and both case-control studies (Botto et al, 2002; Groenen et al, 2004) had the usual recall bias. However the supplementation period was very short, restricted only to the early few months of pregnancy.

The remaining six studies (Tables 1.18. & 1.19.), one observational and five RCTs, looked for an association of single or combination of two or three micronutrients during pregnancy with later child development. Among them all but one (Hamadani et al, 2003) were from developed countries. I could not find any study that used more than three micronutrients during pregnancy to observe the effect on later child development. Two studies used a combination of three micronutrients during pregnancy to see the effect on child development (Table 1.18). In both the studies

iron and folate was common to both treatment and control groups. One looked at the additional effect of zinc (Merialdi et al, 1999) whereas the other mainly looked for vitamin A (Schmidh et al, 2004). The addition of zinc affected foetal behaviour but the validity of this is unknown and the addition of vitamin A (Schmidh et al, 2004) had no benefit. The other four studies observed the association of single micronutrients during pregnancy with later child development, three with zinc and one with iron. Among the three zinc studies one was observational and two were RCTs. The observational study was from Egypt and showed a positive association between maternal zinc intake from animal source and child's motor development (Kirksey et al, 1991, 1994). However neither of the RCTs with zinc found benefits in children's development (Tamura et al, 2003; Hamadani et al, 2002) and one found slightly poorer development compared to placebo (Hamadani et al, 2002). The lowered developmental scores in Bangladeshi infants (Hamadani et al, 2002) suggest the possibility that supplementing with one micronutrient (zinc) may reduce the bioavailability of other micronutrients (copper) in nutritionally vulnerable populations. Thus there is no consistent evidence of a benefit for prenatal zinc supplementation on the offspring's development. The only study that I could locate was on prenatal iron supplementation on child development, but it was reported only in an abstract (Zhou et al, 2004) and showed no effect on the intelligence of children. No other pregnancy micronutrient supplementation study in humans could be traced.

In conclusion, there are very few studies on child development on the effect of micronutrient supplements in pregnancy. No consistent patterns of micronutrient supplementation were observed. The studies varied greatly in doses and combination of micronutrients used, thus making it difficult to comment on. All the RCTs described above were from developed countries except the Bangladeshi one (Hamadani et al, 2002). It is therefore extremely important to know the role of prenatal multiple-micronutrient supplementation on child development to guide policy on recommendations during pregnancy.

Table 1.17. Associations of periconceptional micronutrient supplementation on neural tube defects and child development

Study	Study Design/Exposure	Sample	Procedures/Measures	Results	Comment
Hungary Multiple micronutrients Dobo & Czeizel, 1998 Czeizel & Dobo, 1994	RCT Supplementation group (Elevit Prenatal) - 4000 IU Vit A, 1.6 mg Vit B ₁ , 1.8 mg B ₂ , 19 mg nicotinamide, 2.6 mg Vit B ₆ , 0.8 mg folic acid, 4 µg Vit B ₁₂ , 100 mg Vit C, 500 IU Vit D, 15 mg Vit E, 10mg calcium- phosphate, 100 mg magnesium, 60 mg iron, 1 mg copper, 1 mg manganese, 7.5 mg zinc Trace element group-1 mg copper, 1 mg manganese, 7.5 mg zinc, 7.5 mg Vit C Supplementation Period = (1 month before the planned conception and at least until date of 2 nd missed MP) Follow up = 1 st short term exam of infants at the age of 8 mo (n= 3356) 2nd long term follow-up at 6yrs (n=625 out of 800 invited infants took part- 78 %	4122 mothers with live born child between Jun 1993-May 1994 Supplementation group: n=200 Trace element group: n=200	Anthropometry - weight, height, head and chest circumference Hearing test: Audiological Exam & tympanogram Vision test: Ophthalmological exams & fundoscopy Mental Development- At 2yrs: Developmental Quotient (DQ)by Brunet-Lezine method At 6 yrs: IQ measured by Binet test, Goodenough man drawing test (DrQ) Anxiety- By child version of thematic apperception test at 6 years	Between two groups Anxiety: Significant lower rate of anxiety in both sexes in supplemented group than trace element group Otitis media -significantly higher in supplemented group than trace element group Anthropometric measures- Non significant group difference Ophthalmological- Non significant group difference Audiological exam: Non significant group difference Mental development at 2 yrs: DQ - Non significant group difference Mental development at 6 yrs: IQ- Non significant group difference DrQ – Non significant group difference	Behavioural improvement In supplemented group. Increased incidence OM in multivitamin group may be a chance finding due to multiple comparisons Placebo group was not true placebo with ideal/ inert substance

IU=International unit; IQ= Intelligence quotient; mg=Milligram; OM= Otitis media; RCT=Randomized Control Trial

Table 1.17. (continued) Observational studies of periconceptual micronutrient supplementation on neural tube defects and child development

Study	Study Design/Exposure	Sample	Procedures/Measures	Results	Comment
Netherlands Fe+Mg+Niacin Groenen et al, 2004	Case control Study Exposure assessment of preconceptional food intake by dietary recall	Case infants=Non syndromic Meningo (myelo)cele as case of Spina Bifida (n=23) Control Infants (n=236) without Birth Defect	Food frequency questionnaire ~24 months after conception of index pregnancy. Energy- adjusted mean nutrient intakes compared.	Associations: Low preconceptional intakes of plant proteins, Fe, Mg, and niacin are associated with a 2- to 5-fold increased risk of spina bifida. Mean nutrient intakes were comparable to the Dutch food consumption survey data. Fat, cholesterol, iron, and folate intakes were below the Dutch RDA	Beneficial association between neural tube formation and some periconceptual micronutrient intakes. The study had recall bias and selection bias
Atlanta, USA Non-specific MN Botto et al, 2002	Retrospective case control study Exposure to multivitamin tablets (composition not mentioned) 3 months before pregnancy to end of 1 st trimester of pregnancy > 3 times/ week	Case infants=Non syndromic omphalocele born 1968-1990 (n=72) Control Infants (n=3029) without Birth Defect	Telephone interviews and recall – of mothers about periconceptual use of multivitamins who had live born or still born infants diagnosed as omphalocele.	Associations: Periconceptual multivitamin use was associated with a 60% reduction in the risk for nonsyndromic omphalocele. Any type of multivitamin use has been reported.	Beneficial role of periconceptual multivitamin on birth defect. The study had recall bias and selection bias.
UK Multi-vitamin Holmes-Siedle et al, 1992	Prospective long term follow-up of supplemented group. Supplementation group- with Multi-vitamin Prenavite Forte F (Vit A= 4000 IU, vit D= 400IU, Vit B1=1.5 mg, Vit B2=1.5 mg, pyridoxine=1 mg, nicotinamide=15 mg, Vit C= 40 mg, folic acid= 0.36 mg, iron= 75.6 mg, calcium phosphate=480 mg) Control group- Had no control group, compared with general population (norms). Supplementation period = 1 tab 3 times a day before conception (>28 days) until 2 nd missed menstruation-period Follow-up= up to 7-10 years	Subjects- First 100 live-borns children of supplemented women. (All mothers previously had one or more pregnancies complicated by a neural tube defect)	Birth measures-BW, GA, congenital abnormalities Questionnaire -on clinical, developmental and behavioural information at 2-5 years Telephone and postal questionnaire -on growth, general health, vision, hearing, educational and behavioural status at age 7-10 years	Recurrence of birth defects: Reduced significantly in children of supplemented mothers At long term follow up (age 7-10 years) - Worries, fussiness and fearfulness were found significantly more frequently in 91 children	Beneficial role in reducing birth defects but more neurotic traits in long term Selection bias. Confounded by mothers report.

CLP=Cleft Palate; IU=International unit; MV= Multivitamin; ; mg=Milligram; RDA=Recommended Daily Allowance;

Table 1.18. Effects of 2/3 combined micronutrient-trials during pregnancy and observational studies on child development

Study	Study Design and supplementation	Sample	Measures	Results	Comment
US Zn+Fe+ Folate Merialdi et al, 1999	RCT Supplement group =60 mg iron + 250 µg folate+15 mg zinc. Control group =60 mg iron + 250 µg folate Dose =1 tab daily from 10-24 wks of gestation until 1 week postpartum.	Foetus of 55 pregnant mothers (supplemented & control group) was monitored	Foetal movement Foetal heart rate electronically monitored, at 32 and 36 weeks' gestation	In supplemented group- Foetal movement =significantly higher, number of accelerations, movement bouts, time spent moving, and large movements. Foetal heart rate = significantly higher FHR range with fewer episodes of minimal FHR variability.	+ ve finding Improved fetal neurobehaviour, but the sample size was small. Validity of measurement not known.
Indonesia Vitamin A + Fe + Folate Schmidt et al, 2004	RCT Group1 (Fe+FA+ Vit A) =4800 µg retinyl acetate + 120 mg iron+500 µg folate (weekly supplementation supervised) Group 2(Fe+FA) =120 mg iron+500 µg folate (weekly supplementation supervised) Group 3 (national Fe+ FA) =National iron + folate (daily supplementation not supervised) Dose = 1 tablet per week (group 1 & 2) or per day (group 3), from ~ 18 wks of pregnancy until delivery	376 pregnant (~18 wks) women moderately iron and vitamin A deficient, age 17-35 yrs Group1 (Fe+FA+ Vit A) : n=94 Group 2(Fe+FA) : n=94 Group 3 (national Fe+ FA) : n=88	BSID -at 6 & 12 months	MDI/PDI =No impact of vitamin A supplementation. Weekly and daily iron supplementation had similar MDI/PDI indices.	No benefit 27% loss but sample size was adequate to pick up difference with 90% power.

BSID= Bayley Scale of Infant Development; BW= Birth weight; CLP=Cleft Palate; FFQ= food Frequency Questionnaire;FA= folic acid/ folate; Fe=iron; IU=International unit; MDI= Mental Developmental Index; n= number of subjects; PDI= Psychomotor Developmental Index; RCT=Randomized Control Trial;

Table 1.19. Effects of single micronutrient trials during pregnancy and observational studies on child development

Study	Study Design	Sample	Measures	Results	Comment
Egypt Zn in Diet Kirksey A, 1991, 1994	Observational Study No supplementation Self dietary report and sample weighed by dietician Assessed : 1 week after birth	50 pregnant women out of 121 continued until delivery	Infants 1st week after birth- Brazelton Neonatal Behaviour Assessment Scale (BNBAS) At 6 months- BSID	BNBAS =Infants of mothers who had zinc from animal sources were more attentive (related to habituation cluster) BSID = Maternal plant zinc intake was negatively associated with motor development at 6 month.	Positive association Huge reduction of sample size. Recall bias was there.
Bangladesh Zn Hamadani et al, 2002	RCT Supplement group: 30 mg zinc Placebo =cellulose Supplementation Period- 1tab daily from 4 months' gestation until delivery Assessed around 12 months age	A sub-sample of 168 out of 383 infants from a supplementation study age of 6 months. Supplement group: n=83 Placebo: n=85	BSID-II at 6 and 12 months Behaviour rating- 6 and 12 month	MDI = Significantly lower in supplemented group than placebo group PDI - Significantly lower in supplemented group than placebo group Behaviour- Non significant group difference	Negative effect Marked reduction of sample size
Birmingham, US Zn Tamura et al, 2003	RCT Supplement group (Zinc) =25 mg zinc sulphate Placebo group = not mentioned Supplementation Period- daily from ~190weeks of gestation until delivery FU- ~ 5 years of child's age	294 zinc deficient pregnant women, Supplement group (Zinc): n=173 out of 294 Placebo group: n=182 infants out of 286	Differential ability Scale 6 tests- Visual Sequential Memory, Auditory Sequential Memory, Knox Cube, Gross Motor Scale, and Grooved Pegboard tests-at a mean age of 5.3 years	Neurological development = Non significant group difference	No benefit Possibility of effect to be confounded by poor environmental effect Big loss
Fe Zhou et al, 2004	RCT Only abstract available No detailed information found	391 pregnant mothers were supplemented and 302 participated	IQ at 4 yrs using Stanford Binet Intelligence test	IQ = Non significant group difference. Mean IQ=109 ±11,	No benefit

BNBAS=Brazelton Neonatal Behaviour Assessment Scale; BSID-II Bayley Scale of Infant Development II; Fe=iron; IU=International unit; IQ= Intelligence quotients; MDI= Mental Developmental Index; n= number of subjects; PDI= Psychomotor Developmental Index; RCT=Randomized Control Trial;

1.6. Situation in Bangladesh and study area

In this section we describe the

- Background of Bangladesh
- Nutritional programmes in Bangladesh
- Study area in Matlab and its similarity to other areas of Bangladesh

1.6.1. Background to Bangladesh

Bangladesh is a small country in Southern Asia with an area of 144,000 square kilometers. It is mostly flat plain with some hilly areas in the southeast part. A number of interlacing large rivers which originate from the Himalayas flow across the country to finally unite with the Bay of Bengal (Figure 1.10). The climate is tropical with three main seasons: winter (October to March), summer (March to June) and rainy monsoon (June to October).

The country is overcrowded with population of 144,319,628. Almost 98% population is Bengali and rest is tribal and non-Bengali Muslims. The population is relatively young with 33.1% below 15 years of age, 63.5% between the ages of 15 and 64 and 3.4% age 65 and above (Bangladesh website, 2005). Male-female ratio is 1.05. It is predominantly a Muslim country with 83% Muslim, 16% Hindu and 1% other (Bangladesh website, 2005).

Bangladesh is a poor, overpopulated country with 40% of the population unemployed and 45% below poverty line. The estimated per capita GDP is \$2000. A number of domestic and international efforts are running in parallel to improve the situation. Nearly two-thirds of the population is employed in the agriculture sector, with rice as the single most important product. Natural calamities such as cyclones and floods occur almost every year and cause further economic damage. The literacy rate based on ability to read and write is 43.1%. A recent survey showed that almost one half of the rural mothers in Bangladesh had a BMI less than 18.5 kg/m² (NSP 2001). Population growth is high (2.09%) and birth rate is 30.01 births /1,000 population (Bangladesh website, 2005).

Infant mortality rate is also high with about 62.6 deaths/1,000 live births (Bangladesh Bureau of Statistics, 2002). The rate of low birth weight (LBW) in Bangladesh is 30% (UNICEF, 2001). Though the overall rate of LBW is declining, in different localities in Bangladesh it has been shown to be as high as 50% (Arifeen 2000; Osendarp et al, 2000). This high rate of LBW reflects poor maternal nutrition that transmits from generation to generation.

Figure 1.10. Bangladesh



Typical diet of rural Bangladeshi women: Nutritional surveys over the past decade show that Bangladeshi women, even during pregnancy consume a poor quality diet. According to cultural practice Bangladeshi girls and women eat last in the household and consume least due to the preference for boys and men. The diet of Bangladeshi women is dominated by rice as they can not afford other nutritious foods such as vegetables, fruits and animal products (NSP, 2001). Nutritional Surveillance Project (NSP) data from 6 dietary recall surveys in 57,000 women done in the year 2000 estimated that <15 % of mothers ate lentils, green leafy vegetables, eggs or yellow/orange fruits and vegetables on at least 4 days in a week (NSP, 2001). Their intake of animal source protein was also very low and mainly derived from small fish. Plant source protein mainly came from lentils. Soy oil was the main cooking oil for any type of curry or fries in Bangladesh. The amount of oil consumption by a household is considered as an indicator of diet quality at household level. NSP data in the year 2000 showed that the percentage of malnourished non-pregnant mothers was low in households with relatively more oil consumption (NSP, 2001). Kramer and colleagues (1997) reported that energy intake of rural Bangladeshi (Matlab) mothers was also low, with the average being $\leq 88\%$ of recommended energy intake. Intake showed seasonal variations with the lowest during the pre-harvest season (August to November). Green chilly and spices (turmeric, chilly powder, curry powder etc) are commonly used in all types of curries.

As vegetable and fruit intake is low among rural mothers (unless they grow these in their home garden), their diet is very low in micronutrients (NSP, 2001). Moreover high phytate contents of rice also interfere with the absorption of some micronutrients like iron. A study in rural Bangladeshi women showed that the average dietary iron intake was 6.93 mg/day of which only 0.33mg/day came from animal sources and of the most potentially bioavailable iron came from plant foods. Considering low body iron stores and effects of high phytate intake the bioavailable nonheme iron ranged from 0.80 mg/day (12% bioavailability) to 0.05 mg/day (1% bioavailability). It is far below the daily requirement of 2mg of absorbed iron in adult women (IFPRI report, 2000). National anaemia and vitamin A surveys by NSP reported that 45% of non-pregnant mothers were anaemic and 2.7 % of pregnant mothers had night blindness (HKI/IPHN,

1999a; 1999b). The prevalence of iodine deficiency is also very high in Bangladesh. According to national iodine deficiency disorder survey in Bangladesh (Yusuf et al, 1993) the grade 1 and grade 2 total goiter rate (TGR) was 47.1%. The prevalence was highest in flood prone-areas (50.7%) and the women of child bearing age (14-44 years) were mostly affected.

1.6.2. Nutritional programmes in Bangladesh

Bangladesh has a variety of programmes, some provided by the government and others by various non-governmental organizations (NGOs), aimed at improving the health of mothers and infants. The Bangladesh Integrated Nutrition Programme (BINP) is one such programme which is implemented by the Bangladesh government under its Ministry of Health and Family Welfare in 1995. After completion of its pilot phase a successor programme, the National Nutrition Programme (NNP) was implemented in 2003 to continue the activities. BINP was implemented under two modalities: one is led by the Bangladesh Government and NGO-assisted using the existing health-infrastructure of the Ministry of Health and Family Welfare (MoHFW). The other modality is NGO led and Government of Bangladesh assisted. In the latter, the Bangladesh Rural Advancement Committee (BRAC) took the major lead and was later joined by other NGOs such as PROSHIKA. The majority of funding has come from the World Bank through its International Development Assistance programme (WB-IDA). Some financial support was also provided by UNICEF along with material and technical support.

The overall goal of BINP is to reduce the prevalence of malnutrition in Bangladesh, particularly by improving the nutritional status young children, women and adolescent girls. Direct beneficiaries of the programme are children below two years of age and pregnant and lactating women who have access to the core component of the programme, known as the Community Based Nutrition Component (CBNC). Monthly growth-monitoring systems have been established at all the CNCs for identifying the vulnerable groups for the programme. Community Nutrition Promoters (CNP) monitor growth of all <2year old children, pregnant women from the first trimester of pregnancy,

and lactating women. Children who are severely malnourished or those who show no increment in their weight over 3 consecutive months, and pregnant and lactating women who have a body mass index (BMI) of <18.5, are eligible for enrolment. BINP has 3 main components:

- (1) national level nutrition activities
- (2) intersectoral nutrition programme development
- (3) a community-based nutrition component

The “community-based nutrition component” includes the implementation of various nutrition interventions including nutritional surveillance of young children and pregnant women followed by nutritional supplementation of undernourished subjects. It also includes community empowerment to promote participation, has the following specific objectives:

- To develop capacity at family and community level to monitor nutritional status of individuals.
- To develop capacity at family and community level to care for the vulnerable members particularly the pregnant and lactating women and <2year children.
- To strengthen the quality of maternal and child interventions along with developing the capacity of the government and the community to target the most nutritionally vulnerable groups of the population.
- To develop capacity of the government and the community to link the resources available from local development programmes such as income-generation, household food security, changing food related behaviours, safe-water-access etc with those who are most nutritionally vulnerable to achieve its goal.

The programme is implemented by phases and covers around 60 rural Upazilas (Unions). It covers a total population of 14,828,961 who have access to 13,395 CNCs. Among them 701,959 children under 2 years of age, 153,232 pregnant women and 178,379 lactating mothers are enrolled in the programme. With the programme’s expansion into NNP Phase 1 (of five years’ duration), an additional 79 *thanas* will be added to the original 60 BINP areas including urban areas, totaling 139 *thanas*. NNP Phases 2 and 3 aim to cover the entire country.

1.6.3. Study area

The study area in Matlab, Chandpur is an *upazilla* (sub-district), which has an administrative unit with the lowest-level of management in the health system. It lies in the East-Central plain of Bangladesh in the delta formed by the Meghna and the Ganges rivers and is 53 kilometers southeast of Dhaka, the capital of Bangladesh (Figure 2). The geographic positioning of the centre of Matlab is 23°23'18.53" north latitude and 90°43'12.12" east latitude. The river Dhonagoda, an offshoot of Meghna, flows from north to south, bisecting the study area into two approximately equal halves. Numerous interlacing canals exist here which remain dry in the winter and overflow during the monsoon, causing flooding almost every year. The population of Matlab is approximately 500,000. It is divided into 22 unions (smallest local government body), each having a population about 22,000.

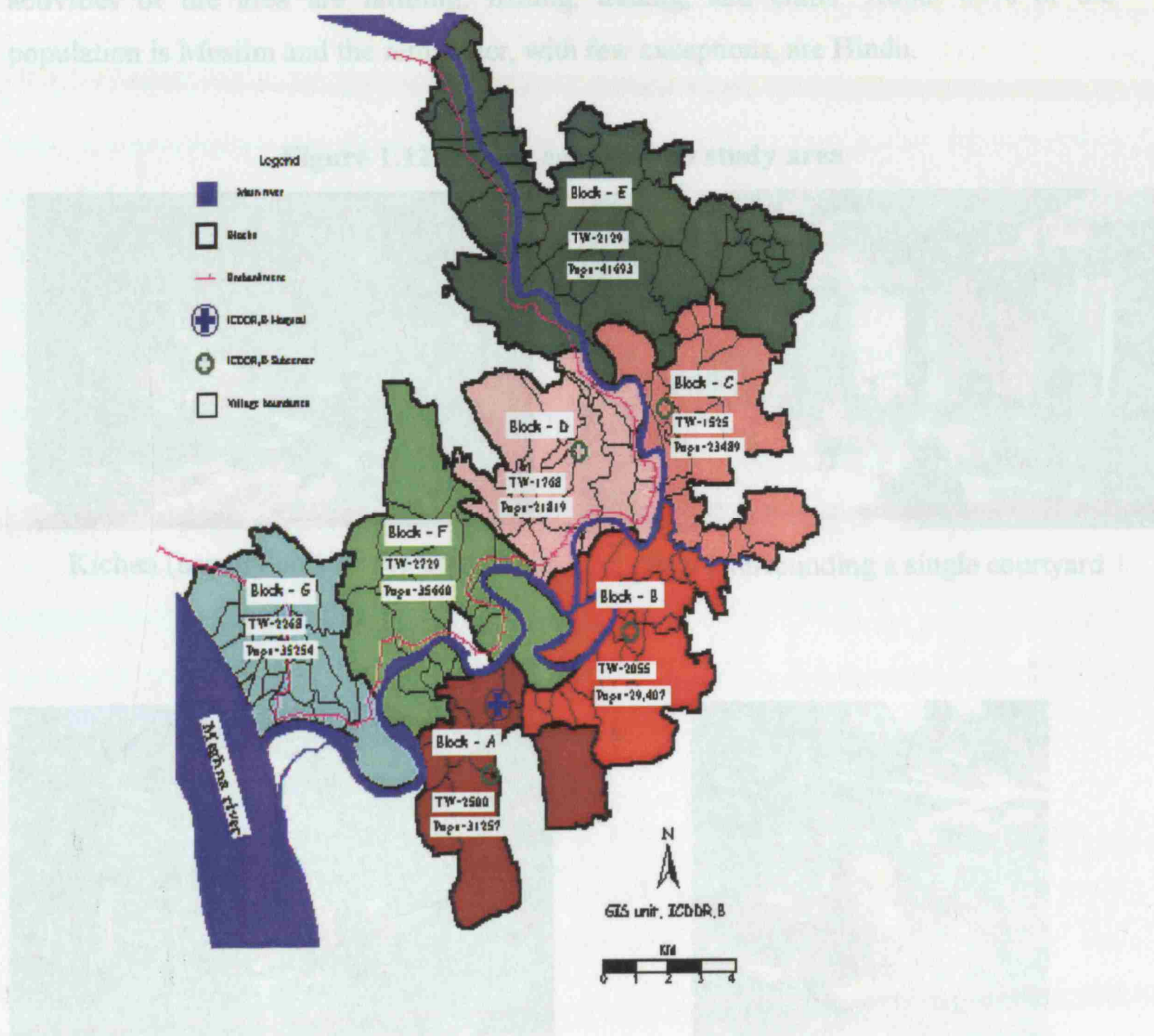
The International Centre for Diarrhoeal Disease Research in Bangladesh (ICDDR,B), Centre for Health and Population Research (CHPR), is an international research institute in Bangladesh which has a study field site of 184 square kilometres in Matlab. The population density of the study area is about 1100/square kilometres and includes a population of 220,000 spreads over 140 villages. The whole area is divided into 2 halves. One half is receiving extensive service delivery from ICDDR,B - "the intervention area", and the other half is getting only government services - "the comparison area".

ICDDR,B has successfully maintained a Health and Demographic Surveillance System (HDSS) since 1966. It is the largest longitudinal demographic data collection system in a developing country and compiles data of vital events such as births, deaths, marriages and migration. Community health workers (CHW) are responsible for collecting demographic data on a monthly basis through household visits. A data storing system has been developed which can trace every individual over time and across studies and databases.

Figure

1.11

Matlab Study Area



Household clearing, washing and bathing
in nearby pond

Child exposed to smoke,
unhygienic play environment

In the intervention area ICDDR,B's service delivery activities include community-based reproductive and child health services. The area is divided into 4 blocks (A, B, C and D).

In the study area members of extended families live in clusters of patrilineally-related individual households called a 'bari' which surround a single courtyard. Each bari is formed with an average of six households which holds about thirty people. These households usually own one or two-room houses mostly with mud floors, interlaced bamboo walls and thatched grass or galvanized iron (tin) roofs. The main economic activities of the area are farming, fishing, trading, and crafts. About 85% of the population is Muslim and the remainder, with few exceptions, are Hindu.

Figure 1.12. Living condition in study area



Kichen (usually outside the house
in summer time)



'Bari', surrounding a single courtyard



Household cleaning, washing and bathing
in nearby pond



Child exposed to unsafe,
unhygienic play environment

In the intervention area ICDDR,B's service delivery activities include community-based reproductive and child health services. The area is divided into 4 blocks (A, B, C and D),

each with a population of 25,000-30,000 and a health sub-centre. These subcentres provided services to 109,573 people in 1998. The total fertility rate is 3.0, with 2,287 live births annually. The sub-centre in each block is run by 2 medical assistants, 1 family welfare visitor and 1 nurse-midwife. All the sub-centres provide general management to sick children and uncomplicated reproductive health services with referral systems to the main primary care facility, operated by ICDDR,B.

In the ICDDR,B-served area, a CHW is identified from the villages and trained to provide reproductive and child health services. A CHW is responsible for about 400 households which they visit every month. They also provide services like immunization, family planning services and treatment for cases of diarrhoea and respiratory infection. They are trained to identify complicated cases (e.g. severe pneumonia, deliveries of infants or reproductive tract infections) and refer them to the sub-centre or primary care facility. They also provide some health education and counsel on reproductive and child health. A study conducted in Matlab showed that women of Matlab were underweight (weight mean \pm 45.2 \pm 5.4 kg) and short (height 149.5 \pm 5.3 cm). They were also thin having an average body mass index (BMI) of 20.2 \pm 1.9 kg/m² (Alam et al, 2003). Pregnancy weight gain was also very low. Women gained around 4 kg weight during the last 20 weeks of pregnancy (Alam et al, 2003), about a half of what is expected among well-nourished women (Institute of Medicine, 1990a).

Maternal diet in Matlab is very similar to the diet of typical rural Bangladeshi women described. Rice is the main food with very little intake of vegetables and fruits. I could locate no published data about the prevalence of micronutrient deficiency in this population. However among our study population around 7% of mother had haemoglobin levels <100 g/l and 50% of mothers had haemoglobin levels <115 g/l at 8-10 weeks of pregnancy. In a subsample of 100 study mothers biochemical parameters showed 9% of them had serum ferritin levels <12 μ g/l, 33% of mother had zinc levels <0.56 μ g/ml and 11% of mothers had serum folate levels <3 μ g/l. In this subsample as many as 49% of mothers were found to be vitamin B12 deficient (B12<200 μ g/l). Data also showed that the percentage of low birthweight has remained static overtime. It was

50% more than 2 decades (Khan et al, 1979) ago and remained at 48% (Alam et al, 2003) until recently. Seasonal patterns of BW variation are observed in Matlab, being the lowest among children born in the winter. Shaharasthi, an *upazilla*, adjacent to Matlab, also has an average BW of 2,513 g with 47% LBW (Shaheen et al, 2000).

In summary, maternal undernutrition and LBW are highly prevalent in Matlab. However, a long-term iron supplementation (60 mg/d) programme in pregnancy has improved Hb concentrations which ranged between 124-127 g/L (Stoltzfus et al, 1998). Exclusive breast feeding in the first 4-6 months is also low in Matlab. Data from Alam and colleagues (2003) showed that 60% of the infants were exclusively breast-fed up to the first 3 months declining to 31% at 6 months. Unpublished 1999 data from the Matlab HDDS show that exclusive breast-feeding in infants declines from 64% at age 0-1 months to 27% at age 2-3 months, and finally 15% at age 4-5 months. The nutritional status of mothers and the incidence of LBW in the study area of Matlab (data collected in the main study) were reasonably similar to statistics for Bangladesh as a whole (Table 1.20.). The exceptionally strong research infrastructure in Matlab and the existing DSS made the place suitable to conduct quality research. As this population is generally stable, long term follow-up of samples would also be also possible.

Table 1.20. Comparison of nutritional information of the study area with national figures

	Matlab	Bangladesh
LBW (%)	28 % ^a	30% ^d
Maternal BMI kg/m ²	20.1 ^a	19.4 ^c
Maternal weight kg	45.4 ^a	43.8 ^c
Maternal height cms	149.7 ^a	150.1 ^c
Haemoglobin g/l ^b (Maternal)	117 ^a	120 ^b

a : Data obtained from present study from 4,289 women collected in 1999-2001 (Unpublished report from Shams El Arifeen, MINIMat annual meeting, 2003)

b: Data obtained from Helen Keller International/Institute of Public Health Nutrition (HKI/IPHN) 1999a

c: Data obtained from Bangladesh Bureau of Statistics, 1998

d: Data obtained from 1995-1999, UNICEF 2001

1.7. Justification of present study

In this study we examined the effect of supplementing pregnant Bangladeshi women with food and multiple micronutrients. The knowledge base is still weak regarding the role of food and multiple micronutrients supplementation during pregnancy on child development of the offspring. A small but moderately consistent beneficial effect of food, particularly of energy on pregnancy outcome, is available from published evidence (Kramer & Kakuma, 2003). Although birth weight showed positive association with subsequent child development (Grantham McGregor et al, 1999), little is reported about developmental outcome of prenatal food supplementation of infants. Findings from food supplementation programmes during pregnancy, with or without lactation, show inconsistent effects on subsequent child development (Rush et al, 1980; Joos et al, 1983).

Rationale for composition of food supplement

The supplement contained roasted rice powder, 80g, roasted pulse powder, 40g, molasses, 20g, and soy-oil, 12 ml which provided 600 Kcal/d of energy and 37.5g of protein. This should easily cover the energy gap if taken 6 days a week, however, it will not cover the micronutrient deficiencies.

Rationale for composition of multiple micronutrient mixture

Recent interest has shifted towards a causal role of multiple micronutrients in determining pregnancy outcome (Ramakrishnan et al, 1999). The beneficial role of some single micronutrients during pregnancy is well established (Grantham Mc-Gregor & Ani, 1999; Fall et al, 2003), but nutritionally deprived populations in developing countries are vulnerable to multiple micronutrient deficiencies and this demand might increase during pregnancy. To address this issue, a body of experts considered supplementing mothers with multiple micronutrients during pregnancy to be the most feasible approach (UNICEF/WHO/UNU workshop, 1999) from a policy point of view.

Although the aim of the 15 micronutrient-mix was to improve birth outcome, there was extremely little empirical evidence from RCTs to support the individual components of

the mix. However a number of issues were taken into consideration when deciding on the precise (UNICEF/UNU/WHO) composition. These included (a) probable deficiency of the micronutrient during pregnancy, (b) other demonstrated beneficial effects on fetal and maternal health, (c) possible interaction with other micronutrients (d) antioxidant properties (e) safety issues (f) cost and (g) ease of taking the supplement.

(a) Probable deficiency of the micronutrient during pregnancy: Iron deficiency is widely reported in undernourished populations. Zinc is highly likely to be deficient in the diet of poor communities who eat little animal food. Vitamin A deficiency has also been reported to be prevalent. Sub clinical deficiency of vitamin B1, vitamin C and vitamin B6 has also been reported in some communities. Vitamin C is secreted in breast milk and the amount depends on maternal diet. Vitamin D was included as women in societies who completely cover themselves with clothes might have deficiencies due to lack of exposure to sunlight (de Onis et al, 1998). (b) Demonstrated beneficial effects on foetal and maternal health: Iron was used as it is reported to reduce maternal anaemia during pregnancy, although its role on birth size was not established. Vitamin A enhances iron metabolism and vitamin C enhances iron absorption. Vitamin A supplementation has also been shown to reduce maternal morbidity during pregnancy (West et al, 1999). The possible beneficial role of zinc in increasing birth weight was considered although the evidence was inconsistent (Shah and Sachdev 2001). Vitamin B12 has role in prevention of anaemia. Riboflavin (B2) was added because of its role on gut integrity and it facilitates absorption of other nutrients. Beneficial properties of iodine and folate on foetal neuronal development are well reported. Although salt iodisation is in place in many countries, iodine was added to reach vulnerable groups who were not covered by iodine fortification programme. The role of vitamin B6 on development of foetal nervous system in animal models was considered. (c) Interactions with other micronutrients: As zinc supplement can interfere in copper absorption, copper was added in the composition. Zinc and iron also compete for absorption so both were included.

(d) Antioxidant properties: Vitamin C was included as an antioxidant as well as an enhancer of non-haem iron absorption. Vitamin E and selenium were included due to their antioxidant properties, in addition selenium has a role in iodine metabolism. (e) Safety issues: For safety issues all micronutrients were given in 1 US/Canadian RDA values except folate that was given at a dose of 400 µg/day. (f) Cost Niacin was kept in the composition, as it was expensive to take it out of the available mixes. (g) Ease of taking the supplement: Calcium and magnesium were excluded as these can increase the tablet size. Finally vitamin K was excluded, as it cannot readily pass through the placental barrier.

As reported in the diet section in Bangladeshi women (section 1.6.1.) there is evidence that these mothers are deficient in iron, iodine (in the 30% of the population not taking iodised salt), zinc, vitamin A and vitamin B12. However their lack of intake of fruit and vegetables and animal products suggests that they are also likely to be deficient in vitamin C, other B vitamins and folic acid. So the UNICEF/WHO/UNU multiple micronutrient mixture should cover the possible co-existing deficiencies in this population. As the dose of each micronutrient was 1 RDA, there is theoretically little chance of toxicity. However, where the intake of energy and protein is well below the RDA we cannot be certain that receiving the RDA of these micronutrients is safe. Also there might be interactions among the nutrients we do not fully understand. Although there were some theoretical reasons for the composition of the UNICEF/WHO/UNU multiple micronutrient mixture, the evidence base regarding the role of micronutrients on pregnancy outcome was inadequate. Thus efficacy trials with the 15 multiple micronutrient mixture were launched in 6 countries (Bangladesh, Pakistan, Nepal, Guinea Bissau, Burkina Faso and Indonesia) to address this question (UNICEF/WHO/UNU workshop, 2004). The main idea was that it might cover coexisting deficiencies of several micronutrients in pregnant mother. The present study concerns a subsample of the Bangladeshi trial. We took the opportunity to address the issue of observing the role of food and multiple-micronutrients during pregnancy on child development.

The proposed research is important for several reasons-

- It will provide causal impact of supplementation on a population with high prevalence of malnutrition and low birth weight, thus constituting a vulnerable group of the population.
- It is the first study that is looking for an impact of prenatal multiple micronutrient supplementation on child development of offspring
- It is the second study with food supplementation in developing country and the first study with both food and micronutrient supplementation during pregnancy that is looking for both combined and individual effect of supplementations on child development.
- The study will provide insight into the already existing government food supplementation programme for pregnant women (BINP, NNP) in Bangladesh and will add information for its further modification if required.
- As the study population is a fixed rural population and there remains a well-established demographic surveillance system in the study area, it will provide a unique opportunity to follow this population for long term follow-up to ask future questions.
- Our work will provide ground work for future developmental studies.
- Information on biochemical markers, ultrasound, immune function, morbidity, dietary intake etc from the work of other investigators in the same study, will allow us to link our findings with many other aspects that might influence child development.

Thus this study will address many pregnancy related nutritional questions that might affect early child development. This study will help us to fill in the gap of knowledge which links maternal nutrition and child development. In turn this information will contribute to the nutritional policy of the Bangladeshi government.

Chapter 2: Hypothesis, Aims and Objectives

2.1. Hypotheses to be tested

The main hypotheses to be tested in the child development component of the MINIMat study are directly related to the randomised study design:

1. Infants of mothers receiving food supplementation during early pregnancy will have better scores than the infants of mothers receiving supplementation during later pregnancy on problem solving ability, motor development and behaviours at 7 months of age.
2. Infants of mothers receiving multiple micronutrients during pregnancy will have better scores than infants of mothers supplemented with only iron and folic acid on problem solving ability, motor development and behaviour at 7 months of age.
3. Infants of mothers with low BMIs ($<18.5\text{kg/m}^2$) at the beginning of pregnancy will show greater benefits from nutritional supplementation.
4. The effects of early food supplementation and multiple micronutrients will be additive.

2.2. Specific Aims

1. To compare the effect of early vs late food supplementation during pregnancy on the child at 7 months of age on the following:
 - Problem solving capacity measured with two “one step problem solving” tests
 - Child's motor development measured on the psychomotor index of the Bayley Scales of Infant Development
 - Child's behaviour during the tests session on Wolke's behaviour ratings that assessed five types of behaviour—approach to examiner, activity level, emotional status, co-operation with test procedures and how much they vocalised during.
2. To compare the effect of multiple micronutrient supplementation with the effect of 30mg iron (Fe) + 400µg folate or 60mg Fe + 400µg folate during pregnancy on the offspring's problem solving capacity, psychomotor development and behaviour at 7 months of age as described above.
3. To determine whether infants of mothers with BMIs below 18.5 kg/m² benefit more than infants of better nourished mothers from the interventions.
4. To determine if there is any additive effect of different interventions during pregnancy on later infant development.
5. To document mean age of attainment of fine and gross motor milestones in the infants over the first 7 months of age.

Chapter 3: Methods

Description of the study

This study was an extension of an ongoing longitudinal experimental study with pregnant women known as “Maternal and Infant Nutritional Intervention at Matlab (MINIMat)” study. The objective of the main study was to evaluate the effectiveness and efficacy of 3 combined public health nutritional interventions (2 during pregnancy and 1 after delivery) with approximately 5,000 undernourished women residing in Matlab, a sub-district of Bangladesh.

The extension concerned the follow-up of a sub-sample of infants born to the mothers in the main study and assessment of the effects of prenatal nutritional intervention (food and micronutrients) on their subsequent development. I did not consider the postnatal intervention (breast feeding counselling) for my thesis, but I did control for it in the analyses. For clarity, the original study will be known as the *main study* and the extension, which is the focus of the thesis, will be known as the *child development component*.

First, I will briefly describe the *main study* then the *child development component* in more detail. The study-site has been described in detail in Chapter 1. In this section both the *main study* and *child development component* will be described under the following headings:

The main study

- ⌚ Study design
- ⌚ Enrolment
- ⌚ Intervention-randomisation and supplementation
- ⌚ Measurements
- ⌚ Staff recruitment
- ⌚ Compliance
- ⌚ Quality control

The child development component:

- ⓐ Study design
- ⓐ Sample size
- ⓐ Measurements
- ⓐ Staff recruitment
- ⓐ Piloting of instruments
- ⓐ Training
- ⓐ Quality control
- ⓐ Ethical consent
- ⓐ Logistical constraints of the study

3.1. The main study

3.1.1. Study design

The ongoing *main study* (continuing its birth cohort) is a longitudinal and randomised study to evaluate the efficacy and effectiveness of two prenatal and one postnatal public health nutritional interventions, designed to improve the health of pregnant women and their offspring. The main outcomes were to be assessed during and after pregnancy.

Using the Matlab HDSS (described in the study area of the previous chapter), pregnant women were identified through regular home visits within 6-8 weeks of conception and randomized prenatally into 2 different types of nutritional interventions. The first intervention was to receive food supplementation either early or late during pregnancy, provided in the “Community Nutrition Centres” (CNCs). In the second intervention each food group was assigned to receive one of three types of micronutrient tablet. Following delivery all mothers of a live born child were randomized for the second time to a breast feeding counselling intervention or health education intervention (Figure 3.1).

3.1.2. Enrolment

Using the HDSS of Matlab, pregnant women who were usually resident in the study area were identified during monthly home visits. Diagnosis was initially made by asking a question about the first day of their “Last Menstrual Period” (LMP). Whenever a woman reported amenorrhea over 2 weeks, she was given a pregnancy test. After giving written and verbal consent to be enrolled in the study, pregnancy was confirmed by “Ultra-Sonogram” (USG) at the local clinic. The LMP date, name and identification of the pregnant women were then communicated to the project office. Women with a confirmed pregnancy of less than 14wks according to USG were then enrolled in the study. Other exclusion criteria included mentally handicapped women (unable to give informed consent) and severely anaemic women with haemoglobin <80 g/L in venous blood taken during the clinic visit. The enrolled women were then randomized to one of the 6 supplemented groups by entering her name into the computerized tracking system. The mothers were then randomized into blocks of 12. There were 6 combinations of treatment, so each treatment group was represented by two blocks to make it more difficult to break the code. Table 3.1 shows the blocks of randomizations and the letters used to label the bottles with micronutrients.

Table 3.1. The 12 blocks into which the women were randomized

		Daily multiple micronutrient supplementation		Daily supplementation with 30 mg Fe and 400 µg of folate (Fe30F)		Daily supplementation with 60 mg Fe and 400 µg of folate (Fe60F)	
Routine food supplementation	Early start	A1	A2	B1	B2	C1	C2
	Usual start	D1	D2	E1	E2	F1	F2

3.1.3. Interventions -randomization and supplementation

Food-supplementation

In the Matlab area, the “Bangladesh Integrated Nutrition Programme/National Nutritional Programme” (BINP/NNP), used to provide food supplements mainly of energy and protein to pregnant and lactating women with a low body mass index” (BMI <18.5 kg/m²). The ingredients of the supplement comprised roasted rice powder, 80g, roasted pulse powder, 40g, molasses, 20g, and soy-oil, 12 ml which provided 600 Kcal/d of energy. It was distributed to eligible mothers through the “Community Nutrition Centres” (CNC) 6 days a week and the women were encouraged to consume it at CNCs under direct supervision of “Community Nutrition Promoters” (CNP). As part of the *main study*, two changes were made in the ongoing programme- 1) the supplement was made available for all enrolled pregnant women irrespective of their BMI and 2) the women were individually randomized to supplementation beginning early or late as follows:

(i) Early food group-

This group received a letter code “E” for early food supplementation and these women were eligible to receive supplementation immediately after diagnosis of pregnancy. On enrolment they were strongly urged to enrol themselves at the local CNC of BINP/NNP for the food supplement programme. The names of the women were also communicated to the CNPs responsible for enrolling the pregnant women in the programme. It was anticipated that they would start food supplementation by the end of the 1st trimester at around 6-13 weeks of gestation.

(ii) Late food group-

This group received a letter code “U” for usual supplementation and was allowed to join the programme at a time of their own choosing. CNPs were not informed about them. No special encouragement to join the feeding programme was given to the women in this group. A previous study in the neighbouring areas showed that the time of their entry (usual start) varied considerably with a mean of 17 weeks of gestation (Shaheen et al, 2000).

Micronutrient supplementation

According to the government recommendation in Bangladesh, all pregnant and lactating women are routinely entitled to receive 60 mg iron and 400 µg folic acid. Prior to this study the supplementation was made available to mothers through CNCs. Mothers were allowed to take those pills home for daily ingestion. As part of the study some changes were made in pill types and pill delivery. All the mothers were randomised into 3 groups to receive one of 3 types of micronutrient supplementation delivered from sub-centres. This supplementation was initiated ~14-15 weeks of gestation during their scheduled visits to the sub-centres. Thus the two food-supplementation groups were divided into three subgroups based on micronutrient supplementation as follows:

(i) Group-1 (Fe30F)-

This group received 30 mg of iron and 400 µg folic acid.

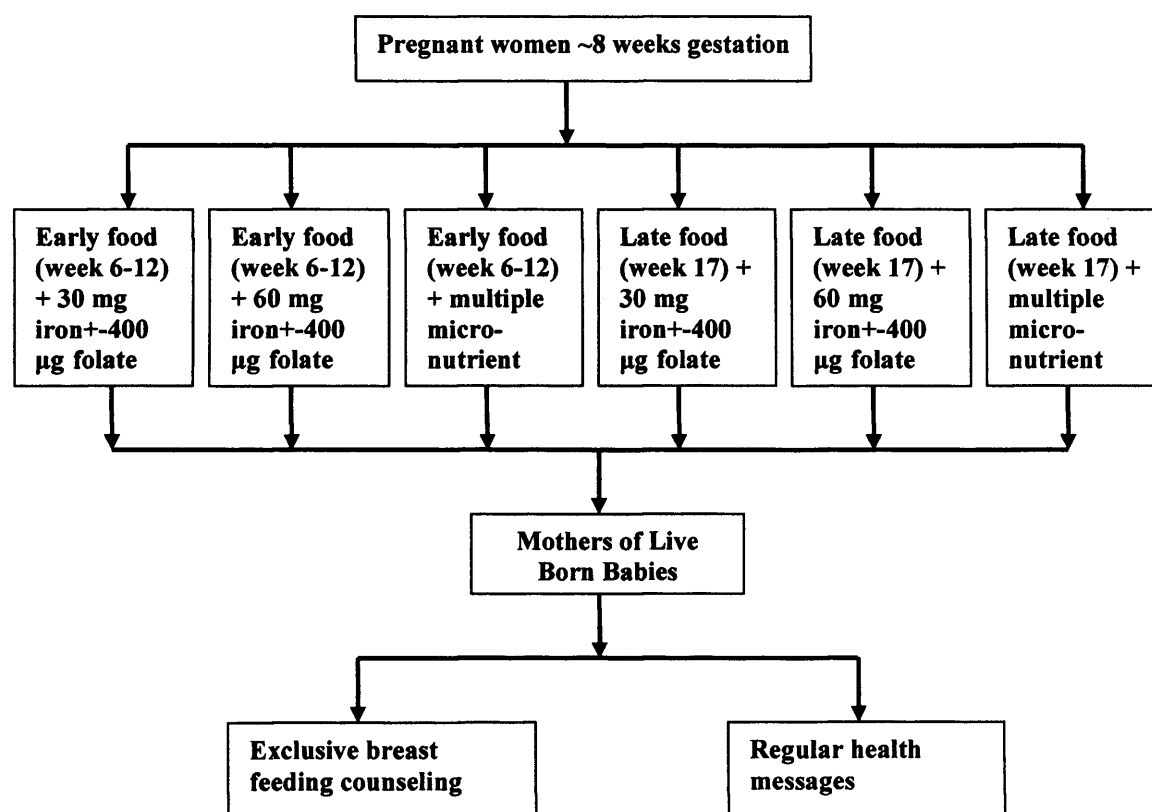
(ii) Group-2 (Fe60F)-

This group received 60 mg of iron and 400 µg folic acid (usual care).

(iii) Group-3 (Multiple- Micronutrients)-

This group received a combination of 15 micronutrients. This combined micronutrient supplement had been developed by UNICEF/UNU/WHO to be used in trials in an attempt to meet the micronutrient deficiencies during pregnancy (UNICEF/UNU/WHO, 1999; 2002). A number of studies in different developing countries (Nepal, Pakistan, Guinea-Bissau, Burkina Faso, China and Indonesia) were initiated using this supplement. It contained iodine, zinc, selenium, copper, Vit A, Vit C, Vit B1, Vit D, Vit B2, Vit E, Vit B6, niacin, Vit B12 in addition to 30 mg of iron and 400 µg folic acid.

Figure 3.1. Schematic description of food supplementation and micronutrient supplementation intervention



Breast feeding

Soon after the birth of a live-born baby, mothers were randomized into two groups as follows:

(i) Breast-feeding counselling group

This group received breast-feeding counselling highlighting the importance and technique of breast feeding soon after birth (< 72 hours of delivery), 7-10 post partum days, and thereafter at months 1, 2, 3, and 5. The breast-feeding counselling was modeled on community-based interventions already shown to be effective in Bangladesh (Haider et al, 2000) and Brazil (Albernaz et al, 1998).

(ii) Health education group

This group received standard health messages on maternal and new-born/child care, hygiene, nutrition etc with the same frequency and intensity as the breast-feeding counselling group.

3.1.4. Measurements

The following measurements were performed by other investigators in the main study that were relevant to the child development study.

Before Birth

(i) Baseline socio-economic status (SES)-

A female interviewer visited the mother's house soon after her enrolment. She collected baseline socio-economic measures including information about family wealth (number of possessions - television, radio, domestic animals, chairs, tables, beds, bicycle, rickshaw etc), and family structure and parental characteristics (mother's age, education, occupation, marital status, reproductive history and frequency of child interaction with the biological father).

(ii) Mother's anthropometry-

All maternal weights were measured with "UNISCALE" electronic scales (UNICEF, 2000) which are accurate to 100 g. Mothers' heights were measured to the nearest 0.1 cm using a stadiometer. Two research assistants were trained to carry out the anthropometric measurements according to standard procedures (WHO, 1983). Measurements were conducted 4 times during clinic visits at 8th, 14th, 19th and 30th week of gestation.

(iii) Monthly follow-up in home-visits-

Monthly home-visits by trained interviewers were carried out throughout pregnancy to assess compliance with the supplement, morbidity and dietary intake. Table 3.2 shows the timing of home visits and the information collected at each visit.

Table 3.2. Schedule of monthly home-visits and information collected at each visit

Timing (Gestational weeks)	Compliance with supplementation	Maternal morbidity	Detailed 24 hour dietary recall	Half-day food frequency
8		+	+	
14	+	+		+
18	+	+	+	+
22	+	+		+
26	+	+		+
30	+	+	+	+
34	+	+		+
38	+	+		+

(iv) Assessments during clinic visits-

After the first sub-centre clinic visit at 8-10 week of pregnancy, each enrolled pregnant woman was requested to make 3 more visits to the clinic at weeks 14, 19 and 30 of pregnancy. Table 3.3. presents the timing of clinic visits and the information collected at each visit.

Table 3.3. Schedule of clinic-visits and tasks performed at each visit

Timing (Gestational weeks)	Ultrasound measure	Physical examination	Blood collection	Urine collection
8	+	+	--	--
14	+	+	venous	+
19	+	+	capillary	+
30	+	+	venous	+

After Birth

(i) Newborn and infant anthropometry - Up to 6 months of age all infant weights (including birth weight) were measured by Seca 835 electronic beam scales (Hamburg, Germany), which were precise to 10 g. Locally manufactured collapsible length boards, precise to 1mm, were used to measure the recumbent length of the infant according to standard procedures (WHO,1983). Head and chest circumference were measured to the nearest 1mm using non-stretchable tape (TALC tape). Infant weights beyond 6 months of age were measured with UNISCALE (UNICEF, 2000).

Infants were measured for all anthropometric measures soon after birth. Then they were measured monthly for weight and length and 3-monthly for head and chest circumferences up to 12 months.

(ii) Postnatal monthly follow-up in home-visits - Monthly home-visits were carried out throughout infancy by trained interviewers to collect information on maternal and infant morbidity and infant feeding.

iii) Assessments during clinic visits - Infants were brought to the sub-centre clinics at the age of 2, 6 and 12 months for thymic examination by ultrasoundogram and blood sample collection (on a sub-sample).

3.1.5. Staff recruitment

Seven types of field workers were used to implement specific study activities as outlined below. These included: paramedics, follow-up interviewers, dietary interviewers, CNC monitors, ultra-sonographers, interviewers trained in qualitative research methods, and counselors.

Staff with different backgrounds (medical professionals, university graduates, anthropologists, nutritionists etc.) were recruited to the study. University graduate female interviewers collected demographic and socioeconomic information. They also conducted all the anthropometric measurements on home-delivered newborn infants. Trained paramedics conducted the clinic examinations, USG, birth measurements when birth occurred at a subcentre-clinic and collection of blood samples.

Female field workers who had completed high school maintained track of the participants, notified births and conducted monthly anthropometric measurements of infants and mothers at household level postnatally from months 1 to 12.

3.1.6. Compliance

Compliance with micronutrient supplementation

For monitoring the micronutrient compliance, special equipment called eDEM® was used.

Ekstrom and colleagues used similar equipment successfully in her micronutrient supplementation studies (Ekstrom et al, 1996; 1999). It consists of a counting device with a small microprocessor which was embedded in the cap of an ordinary pill bottle. It had the capacity to record the time and date of opening and closing the bottle, thus allowing continuous recording of information on compliance until delivery. The bottles were collected and the information in the caps was downloaded onto a computer. The consistency of this information was compared with the count of pills remaining in the bottle from the previous months supply at the time of replenishment of pills during monthly home visits.

Compliance with food supplementation

During monthly home-visits, the interviewers asked the mother a number of questions regarding compliance. Those questions included - number of packets of food supplementation the mother took in the last 30 days, if she shared it with others, how much she ate, when she usually took the supplement etc. This information was later compared with CNP's feeding card records containing information about number of food-packets supplied to the pregnant woman. In addition questions were also asked about intake of food supplements, as part of monthly half-day food frequency questionnaire. Twenty-four hour recalls were recorded three times during pregnancy (table 3.2) to determine how much supplement was consumed and whether substitution occurred.

3.1.7. Quality Control

Data were collected by trained interviewers using structured questionnaires. Many of the feeding questionnaires were adapted from similar, previously used questionnaires in the community and pre-tested on small samples before use.

Refresher training on data collection and anthropometric measurements were carried out every 6 months. Reliability of data on anthropometric measurements were collected according to WHO guidelines (WHO, 1983) prior to the start of the main study and then

after 6 months and one year. Weighing scales were standardised daily with a fixed known weight.

Ongoing monitoring and supervision: Ongoing inter observer reliability was assessed on a 5% random sample by a quality control team on selected questions from a monthly home-visit questionnaire. The team repeated some key questions of the original questionnaire 2 days after the original interview at field level.

3.2. The child development component

3.2.1. Study design

The children born into the study were followed for the first year of life by the MINIMat study group from ICDDR,B. They had a series of measurements including infant growth, morbidity, thymic-ultrasound measure (for immune function), infant feeding and infants' haematological measures (for haemoglobin and micronutrients), as mentioned under the heading of "measures (after birth)" in the description of the main study.

The *child development component* of the study involved a sub sample of infants born to the mothers of the *main study* during in a fixed time period from May 20, 2002 to December 20, 2003. The children were followed from 3 to 7 months of age to determine the effect of the prenatal nutritional interventions on subsequent age of achievement of certain motor milestones and problem solving ability, motor development and behaviour of the infants.

3.2.2. Sample Size

The sample size of the sub sample for the *child development component* was estimated based on the expected improvement in the Bayley motor developmental measures and problem solving test scores of infants born to women receiving various interventions.

Sample size was determined for each group using the following equation of "comparison of two means" (Kirkwood, 1999).

$$n = \frac{(u + v)^2 (\delta_1^2 + \delta_2^2)}{(\mu_1 - \mu_2)^2}$$

Where:

n = Sample required, δ_1, δ_2 = Standard deviations (15 for Bayley motor, 7 for problem solving test), $u=1.96$ (If significance level is 5%), $v=1.28$ (If power is 90%), $\mu_1 - \mu_2$ = Difference between the means (5 points for Bayley motor, 2.5 for problem solving test)

So, for Bayley motor test,

$$n = \frac{(1.28+1.96)^2 (15^2 + 15^2)}{(5)^2} = 189$$

189 infants from each of the 6 intervention groups of women, i.e. a total of 1134, was sufficient to detect a difference of 5 developmental quotient (DQ) points between the groups with a power of 90% at 5% level of significance. With an estimated dropout rate of 30% the sample size increased to around 1475 for total six intervention groups.

And for problem solving test,

$$n = \frac{(1.28+1.96)^2 (7^2 + 7^2)}{(2.5)^2} = 165$$

a total of 990 subjects was sufficient to detect a difference of 2.5 points between the groups with a power of 90% at 5% level of significance. With an estimated dropout rate of 30% the sample size increased to around 1287 for total six intervention groups.

We found 2853 live-singleton babies born during the period May 20, 2002 to December 20, 2003 and recruited them all hoping to see the possible interaction effects among the treatment groups.

3.2.3. Measurements

The child development assessments comprised one test session in the subcentres at the age of 7 months and monthly assessments at home from 3 to 7 months of age (Table 3.4) For the 7 month assessments, trained psychologists who were unaware of intervention groups assessed the infant's problem solving capacity, motor development and behaviour during the session. They were tested in the presence of their mothers in a controlled environment at the Matlab sub-centres. All mothers who travelled to the sub-centres for developmental tests were reimbursed for their travel costs and time. They were also provided with snacks. Infants were given a toy at the end of the tests.

Table 3.4. Schedule for developmental measures at field level

<i>Developmental measures</i>	<i>Age of testing</i>	<i>Location</i>	<i>Testers qualification</i>	<i>Total measures</i>
Two problem solving tests (PST)	7 month 7 day \pm 3 days	Sub-centre clinic	Psychologists University graduates	Once
Bayley motor subscale (PDI)	7 month 7 day \pm 3 days	Sub-centre clinic	Psychologists University graduates	Once
Behaviour ratings (Wolkes')	7 month 7 day \pm 3 days	Sub-centre clinic	Psychologists University graduates	Once
Motor milestone	3 -7 months	At home	Female Field Workers High-school passed	Five times

Using the infra-structure of the main study, the "Female Field Workers" (FFWs) who visited mothers monthly for anthropometric measures and collected various information, conducted the milestone assessment.

Problem solving test (PST)

Two 'one-step problem-solving tests (means-end-problem-solving)' were used to assess cognitive development of infants: *the 'Support' and 'Cover' tests*. The original procedures were described by Piaget (Piaget, 1953, 1955) but the conduct and scoring of the tests were designed by Willatts (Willatts, 1999, 1984).

In these tests infants have to manipulate an intermediary object (a cloth) to retrieve a goal (a toy). The test assesses whether the child intentionally retrieves the toys rather than accidentally while playing. The measurements are described in detail below and the questionnaire used in the study is provided in Annex 2.

(i) Test materials-

Material used for the tests were - a chair for the mother to sit on and a table, a sliding tray, two towelling cloths - 20 cm wide x 30 cm long for the support test and 20 cm square for the cover test, a pool of small plastic squeeze toys (suitable for grasping), a video camera with stand for recording the test session.

(ii) Test procedures-

For both the support and cover test the child had to sit on the mother's lap in front of a table. The height of the table was such that it was up to the waist level of the child, so that the hands of the child were free to play with toys on the table. Before the start of both tests the child was allowed to play for 20 seconds with a cloth and a toy on the tray individually (two presentations for each material) to become familiar with the test materials. In these play-sessions, how far each child could reach to retrieve a toy on the table was assessed and a suitable toy that caught the interest of the child was selected. Five trials were given for each test and each test lasted a maximum of 30 seconds unless the toy was retrieved earlier. Further detailed descriptions of each problem solving test (PST) are as follows.

Support test: In support tests the long towelling cloth was placed on the sliding tray. Next, the selected toy was placed on the furthest end of the cloth while the child was looking at it. Then immediately the tray was pushed in front of the child so that he could pull the cloth to get the toy. To retrieve the toy, the child had to pull the cloth, bring the toy to within reach then grasp the toy. Scoring was based on the 3 reactions - behaviour with the cloth, fixation on the toy (looking at the toy) and behaviour with the toy. A total intention score and the number of trials in which the child showed clear signs of intention while solving the problem were measured later from video-recordings (Fig 3.2.a.).

Cover test: In the cover test the selected toy was hidden under the towelling cloth on the sliding tray out of reach of the child while he/she was watching. Then immediately the sliding tray was pushed in front of the child. To solve the problem the child had to remove the cloth, reveal the toy and finally retrieve it. Scoring is again based on the same three reactions - cloth, fixation and toy while solving the problem (Fig 3.2.b.).

Fig 3.2. Problem solving support and cover test



(a) Support Test



(b) Cover Test

(iii) Scoring-

Both the tests were conducted in a standard way and videoed. The child's performance was scored from a replay of the tape for evidence of intention. Five trials were given for each test but only the the 1st 4 trials were considered for scoring as some children lost interest or tired by the fifth trial. Each behaviour was scored on a 3 point scale, starting from 0 for no evidence of intention, 1 for possible/ambiguous intention and 2 for clear evidence of intention.

'Cloth Behaviour' – *scored until infant contacted or retrieved the toy:*

- Score=0: This behaviour was considered to have no intention if the child failed to contact the cloth or bring the toy within reach or clearly engaged in playing or examining (mouthing, shaking, scratching etc) the cloth at anytime before the toy was within reach.
- Score=1: This behaviour was considered to be ambiguous if the infant pulled the cloth without any play or examination and brought the toy within reach, however before this he began an activity that might be play or examining but he does not carry it through to completion (within 30 sec) or let go of the cloth for more than 1 sec before the toy was within reach.
- Score=2: The infant was awarded a full score for intention in cloth behaviour, if he pulled the cloth without any play or examination and brought the toy within reach. However short breaks in contact of 1 sec or less were allowed provided the infant immediately regained contact. Pauses between movements of cloth of any duration while holding the cloth were permitted. Pulling the toy off the table on the side where the infant was sitting was also awarded full marks.

'Fixation' – *scored until infant contacted or retrieved the toy:*

- Score=0: Fixation behaviour was considered to have no intention if the infant looked away from the toy for more than 2 seconds.
- Score=1: Fixation behaviour was scored as ambiguous if the infant briefly looked away from the toy, but looked back within 2 seconds.
- Score=2: Full intention was scored if the infant was continuously looking at the toy.

'Toy Behaviour' – *scored until the toy is picked up or trial ends (30 sec)*

- Score=0: Toy behaviour was scored as having no intention if the infant failed to contact the toy or touched the toy but made no attempt to grasp it.
- Score=1: The behaviour was scored as partial intention if the infant made an attempt to grasp the toy but could not pick it up.

- Score=2 Toy behaviour was scored as full intention when the child successfully grasped the toy and picked it up.

The scores for each behaviour were totalled to give an intention score for each trial that ranged from 0 to 6, and the trials were averaged to give a mean score. Each trial in which there was evidence of intention (scores of 1 or 2) for all three behaviours (cloth, fixation and toy) was considered to be an “*intentional solution*”. The number of “*intentional solutions*” was also calculated for each test.

Bayley Psychomotor Developmental Index (PDI) :

The psychomotor development of infants was assessed using the revised version of Bayley Scales of Infant Development (BSID-II) (Bayley, 1993). The full Bayley scale has got 2 subscales - mental developmental index (MDI) and psychomotor developmental Index (PDI). For the study I used PDI only (justified below) which measures gross motor and fine motor development. Gross motor items at this age include items such as creeping and sitting, and the fine motor activities include items such as grasping an object, putting pellets through a small hole and scribbling with a pencil etc (Bayley, 1993). This test has been used in previous research in Bangladesh by our group (Hamadani et al, 2001 and 2002; Tofail et al, 2002; Black et al, 2004). The test was administered in the same test session after problem solving test.

Behaviour:

I used a modified version of Wolke’s behaviour rating scales (Wolke D, 1990) for behavioural assessment. I did not use Behaviour Rating Scale (BRS) of BSID-II. Our previous experience showed that it was difficult to get reliability among the testers in BRS of BSID-II (Grantham-McGregor and Hamadani, personal communication). Wolke’s behaviour scale was designed by him for English children and was based to some extent on the Bayley. It had 5 scales with 9 point ratings where each point was distinctly defined. The scales were as follows:

a) *The Approach scale* = This scale measured a child's initial response to the examiner for the first 5 minutes only. At the beginning the tester addressed a few introductory remarks to the child. Then they handed a toy to the child and continued talking to the mothers. The approach is ranged from avoiding=1, to friendly and inviting=9.

b) *The activity scale* = This scale measured how active the infant was during the whole test session (PDI). It was mainly based on gross motor activity of the child and ranged from very still=1, to overactive=9.

c) *The scale for emotional tone* = This scale measured the emotional state of the child during both PST and PDI test. It ranged from unhappy and crying for long periods=1, to radiates happiness=9.

d) *The scale for vocalization* = Vocalization referred to non-crying utterances or to recognizable utterances embedded in crying during test procedures. These included cooing, babbling, consonant sounds or words and ranged from very quiet =1, to constant vocalization =9.

e) *The scale for cooperation*= Cooperation was a measure of how well the infant cooperated with the examiner and complied with her requests. It ranged from resists all suggestions=1, to always complies=9.

Test procedure and measurements of behaviour

We assessed the infants on two different PST tests and the PDI at the same test session and rated behaviour during all three tests. The PST tests were done first and the children met the tester at the beginning of these tests. We therefore scored the 1st behaviour scale “*approach*” (described above) only for the PSTs. During the PST test the testers were instructed to focus the infant’s attention on the toys and not to try and make a relationship with the infants because that might distract them from the toys. Therefore the behaviour scale “*co-operation*” (described above) was not rated during the PSTs but only during the PDI assessment.

The other 3 behaviour scales “*activity*”, “*emotion*” and “*vocalization*” were measured separately for both the tests. However as the infants were not allowed to move around during PST but were held firmly by their mothers, we decided to only consider the “*activity*” rating *during* PDI for analysis. During the PDI the children were free to move around. The score sheet used in the study is provided in the Annex 3.

Motor milestone assessment:

We recorded the child's achievement of certain motor milestones using a combination of mother's reports and observations by the research assistants.

(i) Material used for milestone-

- Toy- 2 cubes, 1 plastic ring, 2 squeeze toys and 1 rattle
- A plastic play-mat
- A one paged structured questionnaire
- A plastic covered pictorial calendar, which was given to mothers at the first visit
- One laminated milestone instruction sheet with all 12 milestones

(ii) Items covered by milestone assessment-

We assessed 12 motor milestones as a part of research work. Six of them were adopted from ‘WHO Multicentre Growth Reference Study (WHO-MGRS)’ and covered milestone of ≥ 6 months old infants. The rest were based on motor items in the BSID-II and covered the younger age group <6 months. As the main study is still ongoing and data on milestones are still being collected, in this thesis I report only on those milestones that had been attained by the infants of my sub-sample at the age of 7 months. Furthermore I will only report findings from mothers' reports and not the observations as all the data from MINNIMat studies are coded and entered onto the computer by a team based at ICDDR,B and are not yet available. Trained field workers visited the homes monthly from 3 months of age until 12 months. They interviewed mothers and observed the child's attainment of different milestones at home. The whole examination took around 10 minutes. The protocol was developed and piloted before

the study began. Validity of mothers' reports was assessed before the study. The score sheet used for milestone is provided in the Annex 4.

We selected 12 milestones (Fig 3.3. and 3.4.) including six gross motor milestones (asterisked) used in the WHO Multicentre Growth Reference Study (MGRS) as follows:

- 1) Holds head erect and steady for at least 15 seconds
- 2) Lifts head and upper trunk on tummy/stomach
- 3) Sits with support
- 4) Picks up toy/cube
- 5) Transfers object from hand to hand
- 6) Sits without support*
- 7) Crawl-
 - a. Moves forward or backward easily by hand and knee crawl, with tummy/stomach not touching the ground (at least 3 in a row)*
 - b. Moves forward or backward easily by any means other than hand and knee crawl (using stomach & arms, buttock & hand/leg, hands & feet etc- at least 3 in a row)
- 8) Pulls self to stand
- 9) Stands with assistance*
- 10) Walks with assistance*
- 11) Stands alone*
- 12) Walks alone*

(iii) Description of testing procedure-

During the 2nd monthly home visit mothers were handed a chart with pictures of each milestone arranged in order of difficulty (Annex 7) and asked to keep a note on the chart when the child attained any of the 1st three milestones. They were also shown how and when to credit the child for a particular milestone. During the next monthly visit the FFW asked the mother if she noticed the attainment of any of the milestones and checked the recording. The FFW then assessed the child for the 1st 3 milestones

according to the sequence in the chart. Mother's reports and FFWs findings were recorded on a questionnaire designed to record milestones attainment at each visit. Then the FFWs ticked the attained milestones on the chart and showed the mother the next 3 milestones she should look for until the next visit. If the mother was illiterate, she was told to ask another literate family member or neighbour to note down the date of attainment on the milestone chart for her. At every visit the FFWs started assessing from the last attained milestone marked on the calendar. Infants' emotional status was also recorded during milestone assessments.

The motor milestones generally follow a sequential pattern. However sometimes the sequence between two or more milestones may be reversed or skipped. So FFWs were instructed not to worry about that. It was often preferable to request the mother or caretaker to elicit the milestone to minimize the child's anxiety to a stranger.

It was expected to be difficult to assess the desired milestones in fussy, sleepy or hungry children. So FFWs were instructed to ensure that the children were happy during the observations and if necessary to take sufficient time to calm them before performing the test. If the child was too sick or irritable, so that it interfered with milestone testing, FFWs were instructed to revisit the child when s/he was settled.

Figure 3.3 . Motor milestones (1st 6 milestones to cover younger age group, < 6 months)



1. Neck Control



2. Lifting head & upper chest



3. Sits supported



4. Picks cube



5. Transfer object



6. Sits unsupported

Figure 3.4. Motor milestones (last 6 milestones to cover older age group, ≥ 6 months)



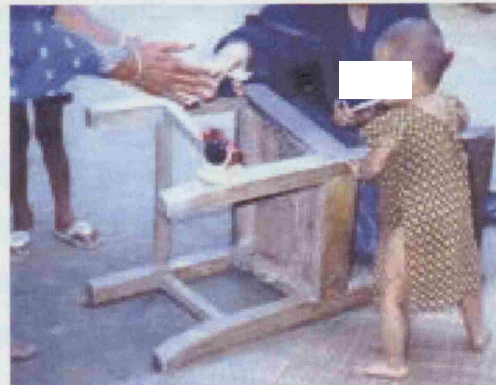
7. Crawl



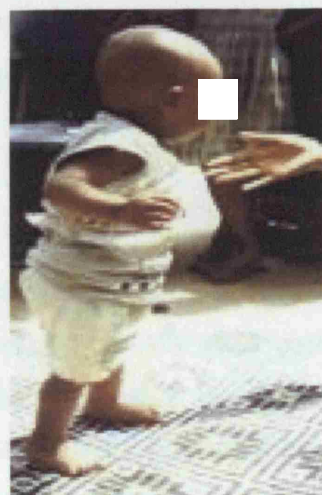
8. Pulls-stand



9. Stands-supported

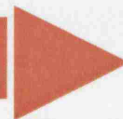


10. Walks -assisted



11. Stands alone

**12. WALKS
ALONE**



Rationale for using the tests described:

(i) Rationale for using problem solving test and Bayley PDI-

Standard infant developmental tests (e.g. Griffith, Bayley, Cattell and Gesell scales) when applied to normal infants during the 1st year of life predominantly measure perceptual and motor abilities, not the more abstract cognitive abilities like information processing and problem solving (McCall, 1979; Willatts et al, 1996). It has been reported that “the findings of these early studies of mental growth of infants have been repeated sufficiently often so that it is now well established that the test scores earned in the first year or so have relatively little predictive validity, in contrast to tests at school age or later” (Bayley, 1970). Thus on many occasions it was shown that the performance on these standardized developmental scales during infancy did not correlate well with performance on the more traditional psychometric intelligence-assessment measures in later childhood (Slater, 1995).

Among these earlier scales, the Bayley Scale of Infant Development (BSID) was a widely used developmental scale that was introduced in 1969. As mentioned earlier, it has got 2 subscales, a mental developmental index (MDI) and a psychomotor developmental index (PDI). MDI is used predominantly to assess cognitive functions and PDI is used to assess motor functions. It has been through repeated revisions and standardizations. New items were added to it based on recent research findings and it had been used in a variety of clinical, educational and research settings. The new revised and re-standardized version (BSID-II) covered age range from 1 to 42 months (Bayley, 1993). It assessed a wide range of developmental measures and has been used in many cultures (Black, 2000).

The BSID-II was well standardized for the United States (US) population with a good test-re-test and internal reliability over short periods for both the mental and motor scales. The content validity (whether the items adequately represent the intended domains of development) was also established by consultation with a panel of experts. The construct validity had been determined by correlations and factor analyses.

Thus the Bayley test had good psychometric properties in the USA, but its use in other cultures, where it had not been standardized, remains questionable.

The Bayley test has not been standardized or validated for Bangladesh. However, we used the Bayley test in 4 separate studies for several reasons (Hamadani et al, 2001 and 2002; Tofail et al, 2002; Black et al., 2004). Firstly, it was the only test we were trained on.

Table 3.5. Bayley scores in different studies done in Bangladesh

Study	Mean MDI	Mean PDI	Age of the child
Hamadani et al, 2001	98.4	102	7 months
	104.8	89.3	13 months
Hamadani et al, 2002	99.3	102.6	13 months
Black et al, 2004a	103.6	108.2	6 months
	104.3	100.9	12 months
Tofail et al, 2003	102.0	101.1	10 month

Secondly, in all 4 studies the infants' mean scores (Table 3.5.) were within the normal range for both scales (MDI for ~6 month > 98, for ~12 months > 103; PDI for ~6 month > 102, for ~12 month > 88). Thirdly, the inter-observer reliability (intra-class) and short term test-re-test stability were good measured in the previous studies.

Thus we decided to use the Bayley test in the study population, but considering the age of testing (7 months) of the children, we planned to use a more appropriate easy test to assess cognition instead of Bayley MDI because:

- (a) Though the mental-development subscale of BSID is considered the gold standard for assessment of mental development among infant and toddlers, it was not originally intended to test intelligence (Bayley, 1969). According to Pollitt (2000), “....the construct intelligence or cognition was not the construct that the developmental scales was expected to tap.” That explains the poor predictive power of these scales below the age of 18 months to predict later intelligence, which showed significant improvement after 18-24 months (Pollitt, 2000).

(b) Though lots of these issues are addressed in the second edition, BSID-II (1993), according to Slater (1994), “In the early months there still seems to be a predominance on perceptual-motor development (at 4 months items include: #36, ‘eyes follow rod’; #44, ‘uses eye-hand coordination in reaching’; #45, ‘picks up cube’). By 12 months what seem to be primarily perceptual-motor items (i.e. #73, ‘turns page of book’; #79, ‘fingers hole in pegboard’; #97, ‘builds tower of three cubes’), are interspersed with items that seem to be measuring Piagetian sensory-motor abilities (#96, ‘find toy under reversed cups’) and language items (#99, ‘points to two pictures’; #100, ‘uses two different words appropriately’)”. So the scale addressed appropriate cognitive items after 2 years of age that includes verbal comprehension, recall of geometric patterns, comparison of masses etc. (Slater, 1994).

Recent research has revealed active cognitive abilities in young infants, such as storing memories and learning. They also reported to have sophisticated visual and auditory perceptual abilities (Goswami, 1998) so evaluation of new assessments of infant’s cognition had been explored. Infant habituation is one such approach which is described as a decrease in attention to a repeatedly presented stimulus and considered as one of the basic tools for assessing learning, memory and cognition in infancy (Willatts et al, 1996). It also reflected individual differences in cognitive function which was moderately stable throughout childhood (Slater, 1995; Colombo et al, 1990). However tests of recognition, memory and habituation in the first 6 months have higher predictive validity but after 12 months the difference is less (Colombo, 1993). It is also reported that some studies failed to demonstrate a significant association between habituation and intelligence scores (Laucht et al, 1994). In addition these tests are difficult to give in large surveys and do not appear to be as sensitive.

The other recently developed test, which has been used in several studies to assess the cognitive behaviour in young infants, was the “problem-solving test” (described above). The ability of young children to solve simple problems (searching for the toy, pulling clothes to get the toy on it) develops rapidly after 6 months. This new skill gradually

improves with age in a very short time period and enables the child to solve more complex problems (combined pulling and searching to get a toy) around 9 months of age. This test is found to be sensitive for nutritional interventions in infants (Willatts et al, 1998; Gardner et al, 2003). It also showed significant correlation with peak-fixation score of visual habituation, indicating better attention control, assessed at the age of 3 months (Willatts et al, 1998).

The other advantages of the one step problem solving tests include-

- Problem solving tests are relatively easy and quick to perform.
- It is easy to train people on this test and maintain quality in larger surveys.
- These tests are scored from videotapes which facilitate ongoing quality control.
- The tests have been used in developing countries (e.g. Jamaica), where they differentiated low birth-weight from normal birth-weight infants, were sensitive to an 8 week intervention at the age of 7 months (Gardner et al, 2003).

For this 7-month old age group we selected 2 “*one-step problem solving tests*” to assess the cognitive behaviour of infants and drop “*mental development*” subscale of BSID-II. The tests were comprehensively piloted before the study began and the detailed instruction for scoring is in Annex 4.

However I chose to use Bayley PDI sub-scale only because motor activities are arguably the predominant activities of infancy and are usually the first skills to be affected by food supplementation. The PDI subscale of Bayley picked up changes following interventions in young infants in Bangladesh (Black et al, 2004a; Hamadani et al, 2001). The test had been used in many other developing countries, for example India (Black et al., 2004b), Indonesia (Idjradinata & Pollitt, 1993; Humphrey et al, 1998), Chile (Castillo-Duran et al, 2001), Brazil (Ashworth et al, 1998) and South Africa (Oelofse et al, 2003). In addition the test fulfilled the requirement of my study that was to compare the development among several supplementation groups in the same community. I aimed to pick up small differences among the different supplementation groups. I did not intend to compare the children's scores with the test norms or with children from other countries. A previous study done in Bangladesh

showed that the PDI at 7 months had moderate but significant correlation ($r=0.40$) with PDI at 13 months age compared to MDI ($r=0.23$) in a normal population (Hamadani et al, 2001). Finally, I was trained in the use and had experience of using the test.

(ii) Rationale for use of behavioural test-

The behavioural ratings had been previously used in Bangladesh with good inter-observer reliabilities and discriminated between effects of zinc treatment (Hamadani et al, 2001; 2002) and effects of stimulation on malnourished children (Hamadani, PhD Thesis paper, 2004). The ratings were also used in Brazil (Ashworth et al, 1998) and were sensitive to differences between low birth weight and normal birth weight babies (Grantham-McGregor et al, 1998).

(iii) Rationale for assessing continuous milestone-

Brain stem mediated primitive reflexes undergo significant evolution during the first year of life. Attainment of gross motor skills indicates not only maturation of the complex neurological development, but also has great impact on children's cognitive development and emotion. The main reason for assessing milestones for the present study was to provide a simple and quick way of obtaining longitudinal measures of development from as early as 3 months. Resources were also limited and the measures are low cost as high school graduates can carry out the assessments. Milestone assessment has been used recently in several nutritional intervention studies and found to be responsive to treatment, for example, in Indonesia (Jahari et al, 2000) and Honduras (Dewey et al, 2001) and showed a difference in nutritionally deficient infants in Zanzibar (Kariger, 2005).

3.2.4. Staff recruitment

I utilised the infrastructure of the main MINIMat study and HDSS of Matlab for conducting our research. In addition to the staff employed on the main study I recruited and trained the following staff for the child development component.

Field Supervisors:

Two senior psychologists and 1 senior field research officer from the Child Development Unit (CDU), ICDDR,B were recruited for training and supervision. All of them were university graduates and had previous experience in this area. I trained 2 psychologists for the problem solving test and all 3 of them on assessment of milestones test. After they achieved satisfactory inter-rater reliability with me, they helped with the field training sessions. We divided into groups and conducted practical training sessions. Later on they assisted me in monitoring the field work and quality control of the child development measures.

Testers (junior psychologists):

Five junior psychologists (university graduates) were recruited who conducted the main developmental tests for the study. Four of them were assigned to one each of 4 blocks and were rotated every 3 months around the blocks. The 5th psychologist rotated to all blocks and conducted any extra tests where there was increased load or where another tester was sick or on vacation. In addition all of them were responsible for monitoring the work and checking the data of FFWs who collected milestone data from the field in their block. They conducted small weekly meeting with other staff in their block to ensure the smooth progress of the overall study.

Female Field Workers (FFWs):

Twenty female field workers (FFW-High school graduates) who were also partly working for the main study were specially trained on milestone assessments. They were responsible for monthly anthropometric measurements for the main study. The milestone component was added to their monthly tasks after they were trained. For the child development component they collected monthly milestone data from the 3rd monthly visit. They also motivated the mothers during home visits to participate in the 7 month developmental tests in the subcentres.

3.2.5. Piloting of Instruments

I piloted two new instruments, the problem solving test and the milestone assessment prior to starting data collection. The BSID-II had been used in this population earlier and piloted before (Black et al, 2004a) so I did not pilot the Bayley motor scale.

Piloting of Problem Solving Test:

Eighty babies aged 6 to 8.5 months were tested. This test was administered for the first time in rural Bangladeshi infants.

My aim for piloting was to determine the following -

- Assess any problems with administering the test
- Determine the relationship between age and scores of infants
- Determine the relationship between birth weight and scores of infants
- Assess test-retest reliability score over 24 hours
- Determine the most appropriate age to test the children in the MINIMat study

Based on the result of piloting I decided that this test was most likely to be sensitive to differences in the children's performance at age 7 months 7 days ± 3 days in the rural Bangladeshi population. I found that half the children attained average intention scores between 2-4 at around this age. (Total possible score =6 least =0)

Result:

- The mean “total support score” and “total cover score” for 4 trials were 10.25 ± 8 and 12.54 ± 6 respectively and were comparable to the mean scores found in other studies (Willatts, 1999; Gardner et al, 2003).
- In the Pilot sample (n=80) I found no relation of test scores (support and cover) with birth-weight and there were no significant differences in total scores between LBW and normal birth weight group. However there was a trend of higher score in the support test for NBW infants compared to LBW infants (11.0 vs 9.0). The birth-weight information of these subjects was collected from the record book.
- Test-retest reliability was also assessed for PST on 15 babies after 24 hours gap.

The correlation co-efficient (kappa= κ) was assessed using *intra class* correlation. Kappa for total intentional score and intentional solution for support and cover tests were-

- | | |
|-------------------------------------|-----------------|
| a) Support total intentional score- | $\kappa = 0.71$ |
| b) Support intentional solution- | $\kappa = 0.64$ |
| c) Cover total intentional score- | $\kappa = 0.75$ |
| d) Cover intentional solution- | $\kappa = 0.79$ |
- I found age “7 month 7 days ± 3 days” to be most sensitive age-of-testing in these rural Bangladeshi infants for conducting the two “one step-means-end-problem-solving” tests. The decision was based on the following information:
 - a) Frequency distribution of scores at different age group.
 - b) Number of children reaching the “ceiling” or remaining in the “floor” at different age.
 - c) Comparing the age of testing for these tests in other countries (Willatts, 1999; Gardner et al, 2003).

Piloting of Problem Milestone Assessment

I piloted the milestone questionnaire on 200 non-study children across the age range of 2 to 15 months from the same locality. The purposes of pilot test were to determine:

- If the mothers understood and followed the instructions.
- If they were capable of understanding the milestone chart and could keep note of the date of attainment of individual milestones on the chart.
- If tests could be performed at home.
- Availability of objects in the homes to enable the test to be conducted.

Based on my findings I modified terms and language of my instructions. I also found that these rural mothers are capable of following the milestone charts. I found it possible to conduct the tests at household level using household objects like chairs, benches etc. but instructed the FFWs to carry blocks, spoons and mats with them.

3.2.6. Training

I conducted an extensive training programme and went through the following steps:

Development of training materials-

I made the following materials for training purposes and on-going guidance.

- A video of the administration of the problem solving tests both in Bengali and English.
- A milestone training manual - with guidelines for assessment of 12 milestones.
- A milestone training video describing how to measure each of the 12 milestones with example of one success and one failure for each measure.
- A pictorial milestone chart - for the mothers with pictures of 12 milestones on it. Field workers were trained on how to guide the mothers to follow the chart and record the date of attainment against each milestone on it.
- A video of 10 children being assessed for each milestone was prepared in order to measure inter-observer reliability from it. There were 5 successes and 5 failures for each milestone.
- A video was also prepared on how to approach mothers at household level.

*****The above materials are in the annex (Annex 6 and 7) and videos are available on request.***

Training procedures and reliabilities-

The training of staff focused on giving them adequate knowledge and conceptual understanding about each measurement (milestones and problem solving tests) and scoring system. The staffs were assessed on how to approach a family, be friendly with them, build rapport and maintain privacy. They were closely observed in how fluent, comfortable and confident they were during the administration of a test. They also taught how to tackle field problems.

(i) Training of supervisors-

As mentioned earlier I trained 3 field supervisors (2 senior psychologists and 1 field research officer). The 2 senior psychologists were trained on PST and all 3 field- supervisors were trained on milestone assessment. The 2 senior psychologists were previously trained on BSID-II, so it was only necessary to obtain inter observer reliability on it.

For the problem solving test, the r value for intra class correlation between me (PI-principal investigator) and 2 psychologists were as follows (Table 3.6)

Table 3.6. Intra-class correlation

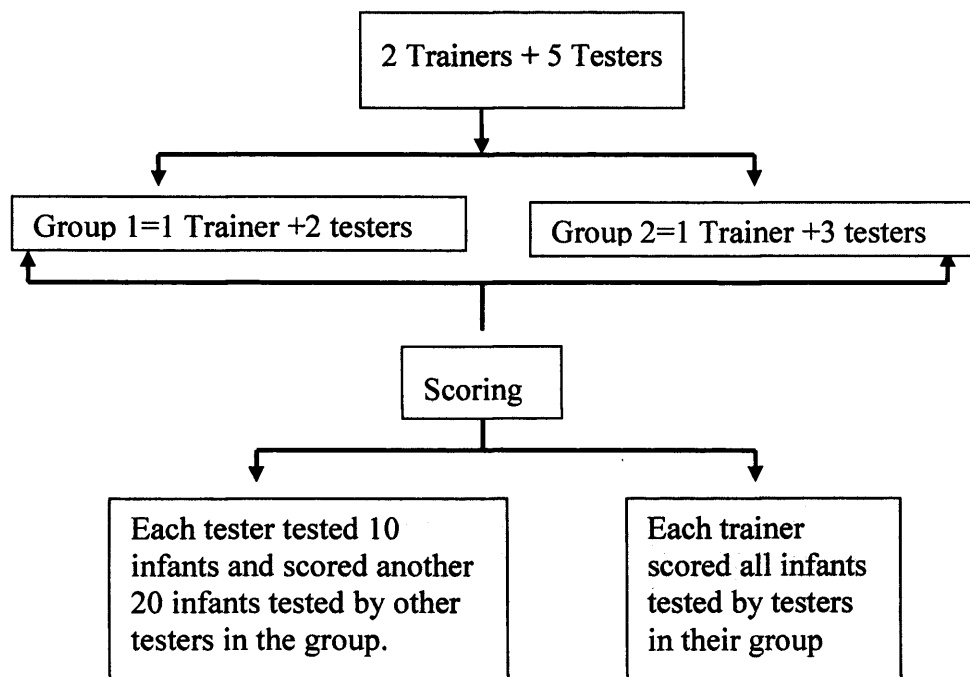
	Intentional solution for support test (n=20)	Intentional solution for cover test(n=20)
PI & Psychologist-1	$\kappa=0.96$	$\kappa=0.95$
PI & Psychologist -2	$\kappa=0.97$	$\kappa=0.96$

For milestones inter coder reliability was > 0.9 as calculated by intra-class correlation on 30 subjects. After having satisfactory agreement between us in all measures, we trained 5 new junior psychologists on PST and BSID-II test and 20 FFWs in assessing milestones.

(ii) Training of testers-

For the Problem Solving Test, each of the 5 testers tested 10 infants and scored 20 additional infants in total until they attained satisfactory agreement with the trainer (Fig 3.5)

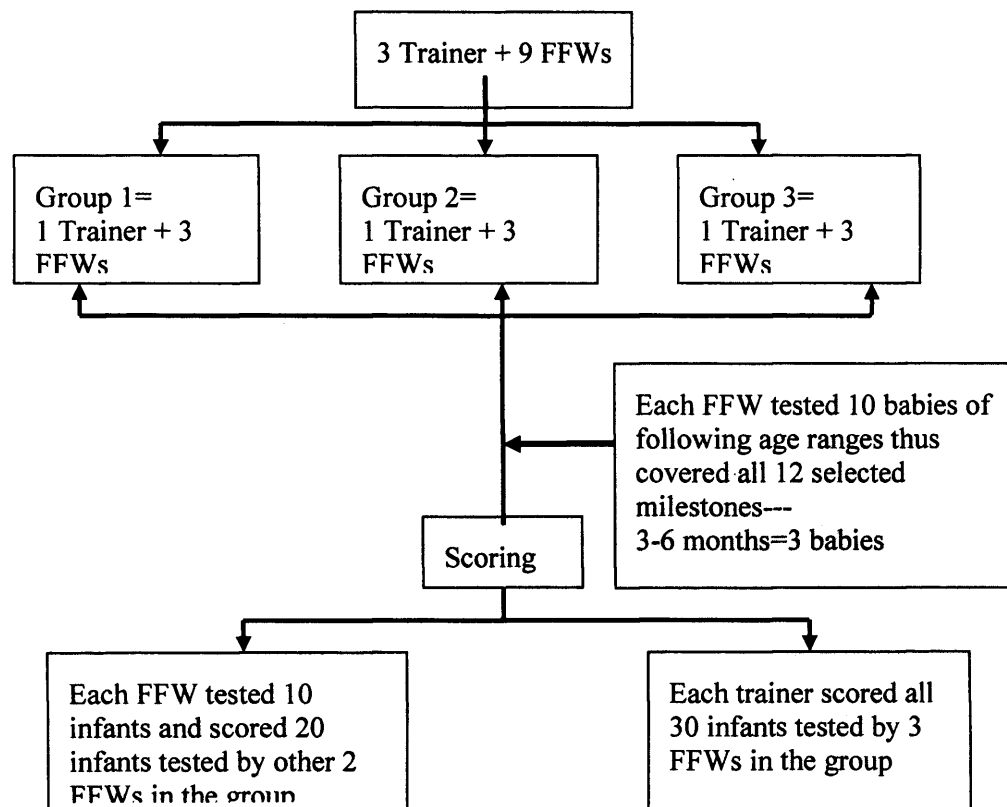
Figure 3.5. Model used for Problem solving Inter-observer reliability.



For milestone assessment we trained 20 FFWs in two batches. The 1st batch of 9 FFWs received 5 days extensive training including a theoretical session, a video session, a role-playing session, open discussion and a practical session in the field.

For inter-observer reliabilities we grouped (1 trainer and 3 FFWs) and tested 30 babies of age range 3-15 months (Figure 3.6). Each FFW tested 10 babies across the age range (3 from 3-6 months; 3 from 7-10 months; 4 from 11-15 months age range) in such a way that they covered all the 12 selected milestones and independently scored 20 babies tested by other FFWs of that group. All started testing for the study after attaining satisfactory inter-observer reliability with the trainers.

Figure 3.6. Model used for conducting milestone Inter-observer reliability in field across the age ranges



3.2.7. Quality control

I supervised whole programme and visited all 4 blocks in the field by rotation - every week for the 1st 6 months then fortnightly for the next 6 months. I worked at building on the strengths and used positive reinforcement to increase the staff's self-confidence. I arranged refresher training for all interviewers and testers every 6 months.

To maintain the quality of tests throughout the study period I took the following measures.

Inter-observer reliabilities:

This was assessed on 20 infants at the beginning of the study. For PSTs the reliability was ($R > 0.95$) for both support and cover tests, for Bayley motor $R = 0.95$ and for all behaviour ratings $R > 0.89$.

Test-retest reliability:

Test-retest reliabilities for developmental tests were conducted before the start of the child development component of the study. Short time interval (24 hour) test-retest reliability for both total support and total cover intentional scores (PSTs) on 15 infants were $R > 0.70$ and for Bayley motor on 10 infants at 7 day intervals was $R = 0.9$.

Ongoing reliability:

To maintain quality of testing ongoing reliability was assessed on 7% of all milestone tests by repeated interviews at field level.

3.2.8. Ethical consent

After giving birth to live born infants all eligible women were again invited to join the child development study. Written informed consent was obtained from them if they agreed to continue the infant part of the study along with their infants. The consent form (Annex 1) described the child developmental measures along with other procedures planned to be done by the other investigators including collection of biological samples, infant thymic ultrasonographic examination, anthropometric follow-up and interviews.

All mother-child pairs were allowed to withdraw from the study at any point without affecting their regular access to ICDDR,B facilities. They were also allowed to withdraw from any particular component of the study without affecting their participation in other components. For example, a mother might refuse to give blood but could still continue child-development tests. Confidentiality of information was strictly maintained.

Ethical consent was obtained from the “Ethical Review Committee” (ERC) of ICDDR,B for all the components of the infant cohort together, and additional consent for invasive procedures was also received from the Research Ethics Committee, London School of Hygiene and Tropical Medicine, UK.

3.2.9. Logistical constraints of the study

The main logistical constraint I faced in this study was limited budget and shortage of staff for the research:

- Due to budget constraints I had to utilize the existing staff in the main study for home visits as they were already over loaded with other tasks for the main study. I therefore faced great difficulty to control, train and regulate their activities.
- As I joined the study after the start of the birth cohort, I therefore had to go through a lot of trouble to get separate space of our own to conduct the developmental tests. Subcentres were health facilities where multiple works were being done everyday- like delivery, health checking, ultrasonogram, biological specimen collection, training, meeting, cooking for staff etc., thus it was not always very quiet. I also had faced a lot of difficulty to get the quietest test-room.
- Data entry was not in our control. The main study group regulated the whole process and they had their own priorities. I had to establish the urgency of getting at least my part of the work entered for my PhD thesis. But as the developmental part was linked with baseline information I could not get all the information collected for the main study, for example, infant morbidity, maternal haemoglobin status at last trimester, infants feeding information etc entered. This is because different investigators were responsible for different data and all data were not entered or cleaned due to lack of manpower (data entry person) and computers.
- Finally, due to monetary constraints I could not hire enough transport to bring the all the mothers to the sub-center for testing their children during the rainy season and flood season in due time.

3.2.10. Analysis

All the data (of main study data and all sub sample data) were entered into an Oracle database using a custom-built Visual Basic data entry programme. The programme had a built-in range and consistency checks. Raw data collected in the four field offices were

directly transferred to the data management section in the main office situated in the main ICDDR,B hospital in Matlab town-centre. Data were entered by four data management assistants under supervision of a full time programmer. The programmer was also involved in developing the data entry programmes for the study. The frequency distribution of all entered data were checked for completeness and consistency. Additionally, a system was developed for all relevant investigators to get access to the interim data sets and undertake exploratory analysis or checks for quality. I extracted my required data from the data pool and analysed them separately using the SPSS-PC version 10. All the variables were rechecked for frequency distribution, missing information and inconsistencies. The outliers were then checked and any suspicion of an error was rechecked and corrected by looking at the raw data.

Socio-economic indices

In order to reduce the data and simplify the analyses, the variables referring to the construction of the house were recoded or scored and added to make a Housing Index as follows: roof, floor and wall were scored as '1' if they were made up of mud, '2' if made of bamboo, straw and/or jute, and '3' if made of tin or cement. Then the added scores were recoded into a dichotomous variable e.g. total score ranging 3-6 (poor housing) recoded as '1' and total scores >6 (better housing) recoded as '2'.

Transformations

Frequency distribution and normality of the variables were checked. Transformations were used to normalise the variables when necessary. The outcome variables, total support and total cover were negatively skewed and they were transformed by square root before use in the analyses.

Difference between the lost and tested children

To examine any differences between lost and tested *children* among the 6 groups: early food and multiple-micronutrients, early food and 30mg-iron + folate (30Fe+F), early food and 60mg-iron + folate (60Fe+F), late food and multiple-micronutrients, late food and 30Fe+F, late food and 60Fe+F we used two- way analyses of variance for

continuous variables. Lost (yes/no) and group (x 6) were the factors. We conducted chi-square test for the categorical variables.

Relationships among variables

Pearson's correlations between the developmental scores, and age and sex were calculated. The relationship between the developmental variables and socio-economic and child variables were examined by partial correlation controlling for age and sex if the developmental variable was significantly related to age and/or sex.

Analyses by intention to treat

The effect of the intervention on each developmental measure was examined using 3-way analysis of variance (ANOVA) analyses with food group (2 levels), micronutrient group (3 levels) and breast feeding counselling groups (2 levels) as factors. As sex was related to support scores we used sex as an additional factor when analyzing this outcome. In addition, age of testing was related to PST and Bayley test scores so we co-varied for age. To examine the effect of low BMI on the treatment effect in further analyses maternal BMI (2 levels) was used as an additional factor in the ANOVAs and only two way interactions were examined. Post-hoc analyses were conducted to examine any significant group difference.

Chapter 4: Results

In this section I am going to present the results of the study in the following order:

- ④ Loss of children from the sample
- ④ Enrolment characteristics of the tested population
- ④ Results of main outcomes
- ④ Correlations with outcome variables
- ④ Intention to treat analysis
- ④ Milestone Reports

The main developmental outcomes were: one step problem-solving tests 'Cover and Support', the Bayley psychomotor developmental index (PDI), and five behaviour ratings during the test session (approach, activity, co-operation, emotion and vocalization). In addition attainment of motor milestones is reported.

4.1. Loss of children from the sample:

To examine any differences between lost and tested children the 6 groups were examined; early food and multiple-micronutrients, early food and 30mg-iron + folate (30Fe+F), early food and 60mg-iron + folate (60Fe+F), late food and multiple-micronutrients, late food and 30Fe+F, late food and 60Fe+F with two-way analyses of variance.

A total of 2853 singleton live births who were born between 20th May 2002 to 20th December 2003 were exposed to prenatal nutritional supplementation and were eligible for enrolment in the child development study. These infants' families were contacted and followed up monthly at home from 3 months of age and tested at the centers around 7 months and 7 days of age. A total 2116 (74%) out of 2853 singleton infants were given the 7 month tests. We tried to test all the infants around "7 months 7 days (217 days) \pm 3 days". However it was a very narrow window of time and it was not always possible for the mothers to maintain the date of visit for family problems. We therefore tested them if

they were available 2 weeks before the targeted age (217 days) and were due to go away later. In case of prolonged absence we continued to look for the children's return until they were 9 months of age. If they were available before 9 months age, we tested them. The reasons for delayed or early tests were mostly due to temporary absence, family problems, rainy seasons (water-locked due to flood), and work at home-particularly during harvest seasons. We assessed almost 50% of the tested infants within 217 ± 3 days and about 90% of the tested infants within 217 ± 15 days. Only 19 infants were tested after 8.5 months. Age of testing was significantly correlated with developmental outcomes, we therefore included all 2116 tested infants in the analysis and controlled for their age. Table 4.1 shows the total number of infants enrolled, tested and lost to follow up at the 7 month test by the 6 treatment groups. The groups did not vary significantly in the proportion of children lost (Chi-squared).

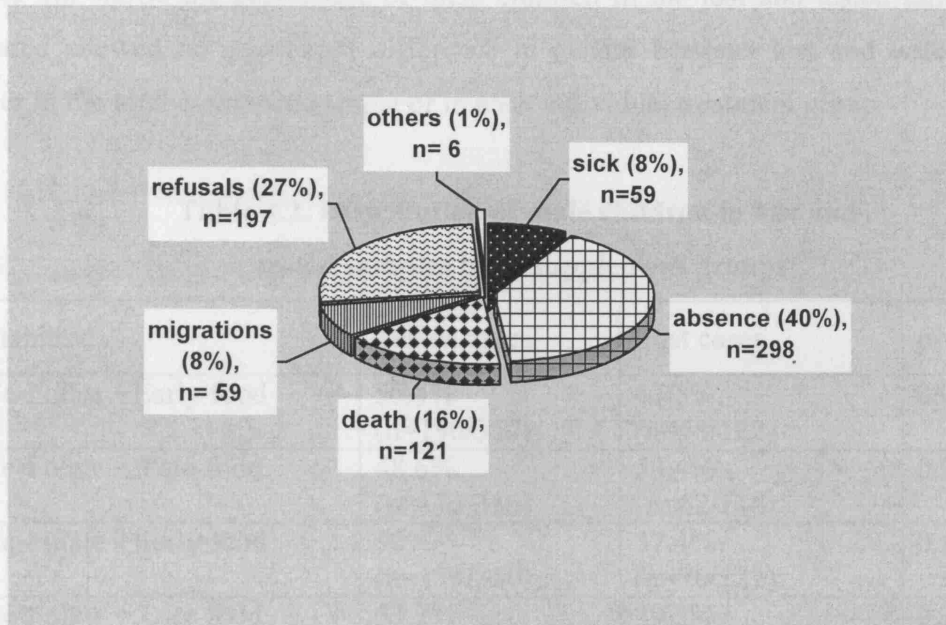
Table 4.1. Number of infants enrolled and given the 7 month test or lost to follow-up by each treatment group

Supplement Groups	Total enrolled	Lost cases	Tested cases at 7 month
30Fe-Folate+ Early food	478	124(26%)	354 (74%)
30Fe-Folate + Late food	473	117(25%)	356 (75%)
60Fe-Folate + Early food	476	126(26%)	350 (74%)
60Fe-Folate + Late food	488	140(29%)	348 (71%)
15 MuMs+ Early food	469	118(25%)	351 (75%)
15 MuMs+ Late food	469	115(25%)	354 (75%)
Total	2853	740	2113

MuMs= 15 multiple micronutrient mix; Fe=iron

Figure 4.1 shows the primary reasons for lost to follow up of 740 subjects. The major causes were prolonged absence from home (40% of total loss), refusal (27% of total loss) and death (16% of total loss). The refusal rate increased when as a part of the main study blood was taken from the infants at six-months of age, beginning from June 2003.

Fig 4.1. Chart showing reasons, number and percentages (within lost sample) for lost to follow-up



In case of refusals, we approached the family at least twice to seek their compliance. Many mothers refused to come for the test because they considered traveling and testing at the subcentre a waste of time, although they were paid. A few mothers initially objected to being videoed during the test. They were reassured when we explained that only the pictures of the infants' performance would be recorded and not their own faces and they were shown some previous videos as examples. However even then one mother continued objecting and so her infant was tested on the Bayley Scales only. One hundred and twenty-one infants (16% of total loss) died before the 7 month testing. Out of them 4% of deaths occurred within 28 days after birth and 12% were between 28 days and the 7 month testing. The reasons for death were varied and the data are not yet available. Fifty-nine mother-child pairs left the study area and could not be traced. Another group of 59 children could not be tested at 7 months due to either maternal or child illness. Among them 37 children reported to be too sick to come for testing on the due date, 21 of them were suffering from diarrhoea and 16 from pneumonia. They remained unavailable for testing up to 9 months of age and were finally dropped from the study.

Two mentally retarded babies were also dropped from the 7 month tests.

Table 4.2 shows the percentage of male children in the lost and tested samples. Chi-squared showed no significant difference in gender between lost and tested children either in the total combined sample or in each individual treatment group.

Table 4.2. Distribution of male children in lost and tested samples by 6 supplement groups

Variables	Tested cases	Lost cases	p-value
30Fe-Folate +Early food	55.4% (n=196/354)	47.5% (n=58/122)	0.08
30Fe-Folate + Late food	48.6% (n=173/356)	53.4% (n=62/116)	0.12
60Fe-Folate +Early food	50% (n=175/350)	57.4% (n=70/122)	0.10
60Fe-Folate + Late food	53.2% (n=185/348)	49.3% (n=68/138)	0.25
15 MuMs + Early food	49.6% (n=174/351)	53.9% (n=62/115)	0.24
15 MuMs + Late food	50.6% (n=179/354)	53.5% (n=61/114)	0.32
Total	51.2% (n= 1082/2113)	52.4% (n=381/727)	0.30

Table 4.3 shows the distribution of three socio economic indicators in lost and tested samples by group. Chi-squared analyses showed that the distribution of all three indicators was not significantly different between lost and found in the combined groups and within each individual group.

Table 4.4 shows parental characteristics of the lost and found cases, both in the total sample and by 6 treatment groups. To examine any difference between lost and found groups, we conducted a two-way ANOVA with the 6 supplemented groups (6 levels) as one factor and “lost and tested” variable (2 levels) as another factor. Mothers’ parity, week of pregnancy at enrolment and hemoglobin level at enrolment showed significant

overall lost-found difference ($p=0.012$, $p=0.017$ & $p=0.018$ respectively). Mothers of children lost to follow up tended to have lower parity (1.13 vs 1.26) and higher hemoglobin level at enrolment (11.8 vs 11.7) than tested children. However the groups did not differ in the characteristics of lost children and there was no lost/found x group significant interaction.

Infants' birth characteristics are shown in Table 4.5. Two-way ANOVA showed that there was an overall significant difference between lost and tested children in gestational age at birth ($p=0.03$) with infants of lower gestational age (39 vs 39.2) being lost. However there was no significant difference among the groups in lost-tested difference and the group x lost-tested interaction was not significant. There were no other differences between lost and tested children in birth weight, length or head circumference.

In summary there was no significant (group x lost- tested) interaction in any enrolment characteristics, which indicates that the differences between lost and found sample were similar across the groups.

Table 4.3. Percentage of three socio economic indicators in lost and tested samples by group at enrolment

Variables		30Fe-Folate +Early food Mean \pm SD (n=354/124)	30Fe-Folate + Late food Mean \pm SD (n=356/117)	60Fe-Folate +Early food Mean \pm SD (n=350/126)	60Fe-Folate + Late food Mean \pm SD (n=348/140)	15 MuMs + Early food Mean \pm SD (n=351/118)	15 MuMs + Late food Mean \pm SD (n=353/115)	Total
% of families with at least one adult with regular employment	Tested cases	33.3%	36%	31.4%	35.3%	37.6%	36%	35%
	Lost cases	33.1%	29.3%	36.5%	36.4%	32.2%	35.7%	35.5%
	In each 6 group p-value	0.53	0.29	0.18	0.45	0.17	0.52	0.41
% of occasional or constant deficit between income - expenditure	Tested cases	20.3%	19.4%	16.6%	19%	19.7%	19%	19%
	Lost cases	20.2%	22.2%	15.1%	21.6%	20.3%	23.5%	20.4%
	In each 6 group p-value	0.54	0.29	0.41	0.30	0.48	0.18	0.21
% of family having a part of their house made of mud	Tested cases	21.5%	20.2%	20.3%	17.5%	25.1%	21.0%	21%
	Lost cases	15.3%	18.8%	19.8%	18.6%	22.0%	20.0%	19.1%
	In each 6 group p-value	0.09	0.43	0.51	0.44	0.3	0.47	0.15

Table 4.4. Socio-economic and parental characteristics between lost and found subjects by 6-supplement groups at enrolment

	Found and lost cases	30Fe-Folate +Early food Mean \pm SD (n=354/124)	30Fe-Folate + Late food Mean \pm SD (n=356/117)	60Fe-Folate +Early food Mean \pm SD (n=350/126)	60Fe-Folate + Late food Mean \pm SD (n=348/140)	15 MuMs + Early food Mean \pm SD (n=351/118)	15 MuMs + Late food Mean \pm SD (n=353/115)	2 way ANOVA, P values
Fathers education in classes	Tested	5.4 \pm 4.6	5.5 \pm 4.7	5.7 \pm 4.5	5.2 \pm 4.5	5.4 \pm 4.8	5.6 \pm 4.4	Lost/tested difference=0.43 Groups difference =0.52 Interaction=0.96
	Lost	5.4 \pm 4.5	5.7 \pm 4.9	5.6 \pm 4.6	4.9 \pm 4.7	5.1 \pm 4.8	5.3 \pm 4.5	
Mothers' education in classes	Tested	4.8 \pm 4.1	5 \pm 4.2	5 \pm 4.2	4.8 \pm 4	4.8 \pm 4.1	5.1 \pm 4	Lost/tested difference =0.68 Groups difference =0.58 Interaction=0.64
	Lost	5.2 \pm 4.2	5.3 \pm 4.1	5.4 \pm 4	4.9 \pm 4	4.9 \pm 4.2	4.6 \pm 4.4	
Mothers' Age in years	Tested	26.22 \pm 5.8	26.36 \pm 5.9	26.43 \pm 6.1	26.15 \pm 6	26.6 \pm 6	25.88 \pm 5.9	Lost/tested difference =0.31 Groups difference =0.19 Interaction=0.79
	Lost	25.3 \pm 5.8	26.04 \pm 5.9	26.32 \pm 6	25.64 \pm 6	26.93 \pm 6.2	25.87 \pm 5	
Mothers' Height in cm	Tested	149.58 \pm 5.6	149.72 \pm 5.2	149.8 \pm 5.2	149.92 \pm 5.1	149.96 \pm 5.1	149.76 \pm 5.4	Lost/tested difference = 0.48 Groups difference =0.22 Interaction=0.28
	Lost	149.33 \pm 5.6	150.36 \pm 5.6	150.12 \pm 5	1.9.34 \pm 5.1	149.97 \pm 5.7	148.66 \pm 5	
Mothers' BMI at enrolment	Tested	20.20 \pm 2.6	19.94 \pm 2.8	20.14 \pm 2.7	19.97 \pm 2.7	20.19 \pm 2.6	20.45 \pm 2.5	Lost/tested difference =0.7 Groups difference =0.62 Interaction=0.13
	Lost	19.78 \pm 2.7	20.45 \pm 2.7	20.06 \pm 2.5	20.26 \pm 2.6	19.89 \pm 2.8	20.19 \pm 2.6	
Parity	Tested	1.26 \pm 1.1	1.25 \pm 1	1.27 \pm 1.1	1.24 \pm 1.1	1.34 \pm 1.1	1.18 \pm 1.1	Lost/tested difference =0.012 Groups difference =0.38 Interaction=0.74
	Lost	0.99 \pm 1	1.21 \pm 1.1	1.1 \pm 1.1	1.1 \pm 1.1	1.2 \pm 1.2	1.1 \pm 1	
Maternal Haemo-globin in g/l	Tested	117 \pm 13	117 \pm 12	116 \pm 13	116 \pm 13	117 \pm 13	118 \pm 13	Lost/tested difference =0.018 Groups difference =0.33 Interaction=0.54
	Lost	118 \pm 13	116 \pm 13	119 \pm 15	118 \pm 12	117 \pm 13	120 \pm 13	

Table 4.5. Infant characteristics of lost and tested subjects by 6-supplement groups at enrolment

Variable	Found/ Lost Cases	30Fe-Folate +Early food Mean \pm SD	30Fe-Folate + Late food Mean \pm SD	60Fe-Folate +Early food Mean \pm SD	60Fe-Folate + Late food Mean \pm SD	15 MuMs + Early food Mean \pm SD	15 MuMs + Late food Mean \pm SD	Difference between Lost and Found P value
Birth weight (gram)	Tested	2700 \pm 440	2684 \pm 437	2716 \pm 379	2664 \pm 380	2694 \pm 381	2717 \pm 388	Lost/tested difference =0.07 Groups difference =0.65 Interaction=0.76
	Lost	2647 \pm 466	2717 \pm 470	2657 \pm 406	2631 \pm 501	2648 \pm 404	2664 \pm 479	
Birth length (cm)	Tested	47.74 \pm 2.4	47.70 \pm 2.3	47.98 \pm 2.1	47.75 \pm 2.1	47.79 \pm 2.2	47.94 \pm 2.2	Lost/tested difference =0.11 Groups difference =0.67 Interaction=0.67
	Lost	47.72 \pm 3	47.79 \pm 2.7	47.86 \pm 2.5	47.48 \pm 2.8	47.62 \pm 2.2	47.4 \pm 2.5	
Birth head size (cm)	Tested	32.43 \pm 1.8	32.4 \pm 1.8	32.5 \pm 1.8	32.42 \pm 1.6	32.4 \pm 1.6	32.58 \pm 1.5	Lost/tested difference =1.0 Groups difference =0.93 Interaction=0.79
	Lost	32.35 \pm 2.3	32.47 \pm 2	32.32 \pm 1.8	32.2 \pm 2.1	32.4 \pm 1.9	32.32 \pm 1.7	
Gestational age at birth (week)	Tested	39.10 \pm 1.8	39.06 \pm 1.7	39.24 \pm 1.6	39.06 \pm 1.6	39.15 \pm 1.9	39.35 \pm 1.5	Lost/tested difference =0.03 Groups difference =0.8 Interaction=0.9
	Lost	39.07 \pm 2.1	39.93 \pm 1.9	38.92 \pm 2.2	38.94 \pm 2.6	39.01 \pm 1.7	39.03 \pm 1.9	
Ponderal Index (weight in gram / length cm ³)	Tested	2.47 \pm 0.3	2.46 \pm 0.3	2.46 \pm 0.3	2.44 \pm 0.3	2.46 \pm 0.2	2.47 \pm 0.3	Lost/tested difference =0.2 Groups difference =0.29 Interaction=0.7
	Lost	2.43 \pm 0.3	2.48 \pm 0.3	2.41 \pm 0.2	2.42 \pm 0.3	0.46 \pm 0.3	2.46 \pm 0.2	

4.2. Enrolment characteristics of the tested population by groups:

Male infants constituted approximately half of the sample size. Only a third of the families had at least one daily wage earner and almost a quarter experienced occasional or continuous income-expenditure deficits in the previous year. A quarter of the families in each group had at least a part of their house (floor or walls) made of mud.

Fifty percent of the parents had primary education (not shown in the table). Mothers were relatively young with a mean age around 20 years and 33% of them were primipara (not shown in the table). Their mean body mass index (BMI) was 20 ± 3 kg/ m² and almost 30% of them had low BMIs (<18.5 kg/m²). Their babies' average birth weight was 2687 ± 400 g and mean gestational age was 39 ± 2 weeks (Table 4.5.). There was no significant difference among the tested samples in the 6 supplement groups in any measured enrolment or birth characteristics.

4.3. Main outcomes:

Table 4.6. and 4.7. show the means and standard deviations of main outcome by groups.

Table 4.6. Means and standard deviation of main problem solving and motor development by 6 treatment group

Micronutrient group	Food group	PST- support (transformed*)	PST- cover (transformed*)	Bayley motor (PDI)
30Fe-Folate n=710	Early food n =354	3.24 \pm 1.3	3.54 \pm 1.1	102.8 \pm 16
	Late food n =356	3.19 \pm 1.3	3.55 \pm 1.1	101.6 \pm 15
60Fe-Folate n=700	Early food n =351	3.21 \pm 1.3	3.51 \pm 1.2	103.4 \pm 15
	Late food n =349	3.11 \pm 1.3	3.56 \pm 1.2	102.2 \pm 15
Multiple-micronutrients n =706	Early food n =353	3.2 \pm 1.3	3.56 \pm 1.2	102.7 \pm 16
	Late food n =353	3.31 \pm 1.3	3.58 \pm 1.2	104.4 \pm 16

* Square root transformation

Table 4.7. Means and standard deviation of main behavioural outcomes by 6 treatment groups

Micro-nutrients group	Food group	Approach	Activity	Vocalization	Emotion	Co-operation
30Fe-Folate n=710	Early food n=354	5.93±1.1	4.71±1.3	2.98±1.6	4.96±1.5	5.05±1.5
	Late food n=356	5.81±1.2	4.73±1.3	3.09±1.7	4.90±1.5	4.93±1.5
60Fe-Folate n=700	Early food n=351	5.95±1.2	4.79±1.4	3.07±1.8	4.89±1.5	4.99±1.6
	Late food n=349	5.84±1.2	4.77±1.1	2.97±1.8	4.82±1.6	4.93±1.6
Multiple-micronutrients n=706	Early food n=353	5.85±1.2	4.69±1.4	2.91±1.7	4.72±1.5	4.80±1.6
	Late food n=353	5.82±1.2	4.84±1.4	3.10±1.7	5.00±1.5	5.04±1.5

Compliance

It was monitored using a number of different sources of information, I had information only about the total number of food packets consumed by mothers during pregnancy for food-supplements. The mean±sd number of food-packets consumed by the mothers until delivery was 78±41 (early food group 92±41 and late food group 63±36). Similarly for micronutrient supplementation, compliance was monitored using information downloaded from a counting device embedded in the pill-bottle-cap and correlated with the count of remaining pills in the bottle from the previous month's supply (detail description in methods section). The mean±sd pill intake by the mothers until 30 weeks gestation was 77±34 (69% of expected). Later consumption is not available at the present.

4.4. Correlations with outcome variables:

As all the developmental tests could not be performed at a fixed age, we examined whether age was correlated with the scores of the 3 outcome variables. The relationship with sex was also examined (Table 4.8.). Age had a small but significant positive

correlation with support ($p<0.01$), cover ($p<0.05$) and a slightly higher negative correlation with PDI ($p<0.001$). Sex was only correlated with the support test scores ($p<0.05$). Therefore when looking for a treatment effect age was controlled for in analyses of all three test scores and sex in analysis of the support test scores.

Table 4.8. Correlations of developmental outcomes with age at assessment and sex

Outcome Variables	Age	Sex
Total Support score	0.07**	-0.05*
Support full intention	0.08***	ns
Total Cover score	0.05*	ns
Cover full intention	0.09***	ns
Bayley PDI	-0.21***	ns
Approach	ns	ns
Activity	ns	-0.05*
Emotional tone	ns	-0.06**
Vocalization	ns	ns
Co-operation	-0.06**	-0.07**

* <0.05 , ** <0.01 , *** <0.001

Table 4.9 shows the inter-correlations among the 7 months' developmental and behavioural measures controlling for age at test. As expected the total problem solving scores were highly correlated with the full and partial intentional solutions for both cover and support tests. The two problem solving scores also had moderate correlations with each other ($r=0.58$). Problem solving scores were only moderately correlated ($r=0.30$) with PDI, which was expected as PDI measures motor skills while problem solving is dealing with cognitive function

PDI was correlated with all the behaviour ratings and showed the highest correlation with activity ($r=0.54$). It is likely that motor ability partly determines activity level at this stage of development (Gardner et al, 1999). Both problem solving tests had low significant correlations with each behaviour rating (from $r=0.32$ to 0.18). All behaviours correlated with each other. The highest correlation ($r=0.81$) was between co-operation and emotional tone, suggesting that these two ratings are capturing the same behaviour to some extent.

Table 4.9. Partial correlations among the developmental and behavioural measures controlling for age

Variables	Total Support	Support full & partial intention	Total Cover	Cover full & partial intention	Bayley motor (PDI)	Approach	Activity	Emotional tone	Vocalization
Support full & partial intention	0.95***	--	--	--	--	--	--	--	--
Total Cover	0.58***	0.59***	--	--	--	--	--	--	--
Cover full & partial intention	0.49***	0.50***	0.93***	--	--	--	--	--	--
Bayley PDI	0.32***	0.28***	0.28***	0.25***	--	--	--	--	--
Approach	0.28***	0.29***	0.26***	0.24***	0.17***	--	--	--	--
Activity	0.32***	0.31***	0.26***	0.25***	0.54***	0.32***	--	--	--
Emotional tone	0.26***	0.27***	0.27***	0.27***	0.25***	0.39***	0.52***	--	--
Vocalization	0.18***	0.19***	0.18***	0.18***	0.18***	0.26***	0.43***	0.40***	--
Co-operation	0.28***	0.28***	0.27***	0.28***	0.26***	0.40***	0.51***	0.81***	0.37***

Table 4.10. shows partial correlations of family characteristics at enrolment with the problem solving test scores and PDI, controlling age of testing. Both problem solving tests had very low but significant correlations with assets, housing and income and most parental characteristics except parity and maternal haemoglobin level at enrolment and in the case of cover scores maternal height. Fathers' education had the strongest relationship (r ranges from 0.07 and 0.12) with the problem solving scores. In contrast, PDI had fewer significant correlations with socio-economic variables.

The associations with father's education and all three child development measures were significant in univariate correlations but the association with mother's education was only significant with problem solving tests. This is surprising but it may be that the fathers are the main earner in the families and the correlations with motor development indicate better economic conditions in households with more educated fathers.

**Table 4.10. Partial correlation of all baseline characteristics
with main outcome variables**

Variables	Total support score	Total cover score	PDI
Enrolment criteria (n=1979)			
Asset	0.08**	0.08**	0.05*
Housing	0.07**	0.08**	ns
Income	0.05*	0.06**	ns
Mothers education	0.06*	0.07**	ns
Fathers education	0.09***	0.12***	0.07***
Mother's age	0.08**	0.08***	0.05*
Mother's BMI	0.06**	0.05*	0.09***
Mother's height	0.04*	ns	ns
Mother's parity	ns	ns	ns
Maternal Hb	ns	ns	ns

p* <0.05 ; p** <0.01 ; p*** <0.001

Table 4.11. shows partial correlations with child's birth size and current nutritional status with the problem solving test scores and PDI, controlling age of testing. All scores were positively correlated with birth weight, height and head circumference, but only PDI was correlated with ponderal index. There was a tendency of PDI to have slightly higher correlations with birth sizes than problem solving tests. Thus overall findings (Tables 4.10 & 4.11) suggest that PDI is more related to biological factors and problem solving tests are more related to socio-economic status.

Table 4.11. Partial correlations between child size at birth and 7 months with the problem solving test scores and PDI.

Variables	Total cover score	Total support score	PDI	Birth weight	Birth length	Birth head circumference	Ponderal Index	7 months height-age	7 months weight-age	7 months weight-height
Total support score ψ	0.58***	--	--	--	--	--	--	--	--	--
PDI ψ	0.28***	0.32***	--	--	--	--	--	--	--	--
Birth weight Θ	0.12***	0.12***	0.24***	--	--	--	--	--	--	--
Birth length Θ	0.14***	0.13***	0.19***	0.74***	--	--	--	--	--	--
Birth head circumference Θ	0.09***	0.11***	0.15***	0.65***	0.62***	--	--	--	--	--
Ponderal Index Θ	ns	ns	0.10**	0.46***	-0.24***	0.15***	--	--	--	--
7 months height-age \S	0.12***	0.14***	0.19***	0.51***	0.57***	0.37***	ns	--	--	--
7 months weight-age \S	0.11***	0.13***	0.15***	0.48***	0.42***	0.32***	0.13***	0.67***	--	--
7 months weight-height \S	ns	ns	ns	0.11***	ns	ns	0.20***	-0.13***	0.65***	--
6 month head circumference Ξ	0.10***	0.13***	0.08**	0.39***	0.40***	0.41***	ns	0.37***	0.45***	0.18***

p* < 0.05; p** < 0.01; p*** < 0.001, \S n = 1617, Θ n = 2004, ψ n = 2113, Ξ n = 1394

Among the birth measures, birth weight and birth-length are highly inter-correlated ($r=0.74$) and both are moderately correlated with birth head-circumference ($r=0.65$ and 0.62 respectively). Ponderal index at birth was positively correlated with birth-weight and head-circumference but negatively with birth-length. Birth weight, length and head circumference were positively and moderately correlated with 7 month height-for-age and weight-for-age and 6 month head-circumference (r =ranging from 0.32 to 0.48). Whereas 7 month weight-for height showed only small but significant association with birth weight. Ponderal index at birth showed small but significant positive correlation with 7 month weight-for-height and weight-for-age but none with 7 month height-for-age or 6 month head-circumference.

4.5. Results of the main outcomes (treatment effect):

4.5.1. Main intention to treat analysis:

There were small significant differences between lost and tested samples in that mothers of children lost to follow up tended to have lower parity, later pregnancy week at enrolment and higher hemoglobin level at enrolment than tested children. Infants of lower gestational age also tended to be lost. However, the groups were not significantly different in the proportion of children lost or type of children lost. Also there were no differences in enrolment characteristics among the 6 treatment groups. We therefore only controlled for age at test (and sex of infants in the support test) in the first series of analyses of treatment effects.

Treatment effects were examined using intention to treat analyses with micronutrient interventions (3 levels) and food (2 levels) as factors. In the main study after birth there was a third nutritional intervention - breast feeding counselling. We therefore controlled for any effect of this intervention by using it as a third factor (breast-feeding counselling yes=1, no=2) in the analyses. Therefore three-way analysis of variance (ANOVA) was conducted controlling for age. Sex is used as an additional factor only for a support test.

Table 4.12. Results of analyses of variance showing effects of treatments on the problem solving tests controlling for age and sex (for support test only).

Developmental Tests	Variables	Type III Sum of Squares	df	Mean Square	F	Sig.
Support total score (transformed) Adjusted R squared = .006	Age of testing	19.08	1	19.08	11.41	0.001
	*Sex of child	8.57	1	8.57	5.13	0.02
	Food group	0.07	1	0.06	0.04	0.85
	Micronutrient group	3	2	1.5	0.9	0.41
	Breast feeding group	0.06	1	0.06	0.03	0.85
	Food x Micronutrient group	4.29	2	2.14	1.28	0.28
Cover total score (transformed) Adjusted R squared = .001	Age of testing	7.79	1	7.79	6.06	0.01
	Food group	0.32	1	0.32	0.25	0.62
	Micronutrient group	0.31	2	0.15	0.12	0.89
	Breast feeding group	1.74	1	1.74	1.35	0.25
	Food x Micronutrient group	0.15	2	0.07	0.06	0.94
Support combined partial-total intentional solution Adjusted R squared = .005	Age of testing	15.29	1	15.29	6.6	0.01
	*Sex of child	18.9	1	18.9	8.15	.004
	Food group	0.51	1	0.51	0.22	0.64
	Micronutrient group	6.35	2	3.18	1.37	0.26
	Breast feeding group	0.36	1	0.36	0.15	0.70
	Food x Micronutrient group	3.16	2	1.58	0.68	0.51
Cover combined partial-total intentional solution Adjusted R squared = .002	Age of testing	1.91	1	1.91	1.12	0.29
	Food group	0.45	1	0.45	0.26	0.61
	Micronutrient group	0.69	2	0.35	0.20	0.82
	Breast feeding group	1.35	1	1.35	0.79	0.37
	Food x Micronutrient group	1.10	2	0.55	0.32	0.72

*Sex: male=1, female=2

There was no main effect of the interventions on any of the problem solving test scores (Table 4.12). Sex was used as a factor for both the support test scores. The male-infants performed significantly better than the females in both support total scores (3.27 ± 1.3 vs 3.14 ± 1.3 , $p=0.02$) and support combined partial + total intentional solutions (2.76 ± 1.5 vs 2.56 ± 1.6 , $p=0.004$).

It is possible that male infants are better looked after and played with more than female infants according to the cultural practice of the society of favouring males. This could explain the independent sex effect in favour of male infants. It is a common belief of the society that the male child will be more economically productive in the future and look after the parents. So they were usually offered better food and better care (NSP, 2001; Brown et al, 1994)

The models were significant for both support total scores ($p=0.007$) and support combined partial + total intentional solutions ($p=0.012$) but not significant for any of the cover tests.

Table 4.13. Results of analyses of variance showing effects of treatments on Bayley psychomotor index controlling for age

Developmental Tests	Variables	Type III Sum of Squares	df	Mean Square	F	Sig.
Psychomotor Index (PDI) Adjusted R squared = 0.045	Age of testing	23540.5	1	23540.5	100.13	<0.001
	Food group	21.42	1	21.42	0.09	0.76
	Micronutrient group	969.91	2	484.95	2.06	0.13
	Breast feeding group	2.09	1	2.09	0.01	0.93
	Food x Micronutrient group	942.99	2	471.49	2.01	0.14

The results of ANOVA of the PDI are given in Table 4.13. There was no significant main effect of any intervention. The model was highly significant ($p<0.001$).

We examined the effect of interventions on the 5 behaviour ratings (approach, activity, emotion, vocalization and co-operation) using similar ANOVAs to those used above. There was no significant main effect of supplementation on any behaviour (Table 4.14). Sex of children was used as a factor in the analysis for activity, co-operation and emotion scores and male-infants performed significantly better than female infants in all these behaviours. The model was significant for emotion ($p=0.04$) and co-operation ($p=0.003$) tests but not significant for the other behavioural tests.

Table 4.14. Results of ANOVA showing effect of the supplementations on behaviour ratings

Developmental Tests	Variables	Type III Sum of Squares	df	Mean Square	F	Sig.
Approach Adjusted R squared = -.001	Food group	4.03	1	4.03	2.80	0.09
	Micronutrient group	1.17	2	0.58	0.41	0.67
	Breast feeding group	0.16	1	0.16	0.11	0.74
	Food x Micronutrient group	1.02	2	0.51	0.36	0.7
Activity Adjusted R squared = .000	*Sex of child	8.39	1	8.39	4.41	0.04
	Food group	1.25	1	1.25	0.66	0.42
	Micronutrient group	1.18	2	0.59	0.31	0.73
	Breast feeding group	0.4	1	0.39	0.21	0.65
	Food x Micronutrient group	2.7	2	1.35	0.71	0.5
Emotion Adjusted R squared = .004	*Sex	15.65	1	15.65	6.55	0.01
	Food group	1.53	1	1.53	0.64	0.42
	Micronutrient group	2.2	2	1.1	0.46	0.63
	Breast feeding group	1.33	1	1.33	0.56	0.46
	Food x Micronutrient group	13.55	2	6.78	2.83	0.06
Vocalization Adjusted R squared = .001	Food group	2.28	1	2.284	.787	0.38
	Micronutrient group	0.3	2	.149	.051	0.95
	Breast feeding group	0.08	1	.076	.026	0.87
	Food x Micronutrient group	8.1	2	4.048	1.395	0.25
Co-operation (transformed) Adjusted R squared = .007	Age of testing	16.75	1	16.75	7.01	0.008
	*Sex of child	23.85	1	23.85	9.97	0.002
	Food group	0.37	1	0.37	0.15	0.7
	Micronutrient group	1.14	2	0.57	0.24	0.79
	Breast feeding group	0.88	1	0.88	0.37	0.54
	Food x Micronutrient group	12.26	2	6.13	2.56	0.08

*Sex: male=1, female=2

4.5.2. Further analysis considering maternal BMI

One of our hypotheses was that women with low BMIs in pregnancy would benefit more than other women. Accordingly we ran a second series of analyses of variance adding BMI (BMI <18.5 kg/m² yes/no) as an extra factor.

Problem solving tests:

In analyses of the “support total score” BMI was significant ($p=0.007$) with higher scores with higher BMI and the interaction between BMI and food was also significant ($p=0.027$). The findings for the “cover total score” were very similar with BMI being significant ($p=0.048$) with higher scores with higher BMI and the interaction between BMI and food ($p=0.047$). The analyses of the “full and partial intentional scores” gave similar results for the support test where BMI was significant ($p=0.004$) and the interaction between BMI and food was significant ($p=0.02$). For the “cover test intentional scores” BMI was not significant ($p=0.1$), but the interaction between BMI and food ($p=0.047$) was significant (Table 4.15). The models were significant for both support tests ($p<0.001$) but were nearly significant for total cover test ($p=0.065$) and not significant for cover full and partial intentional solution.

These results indicate that infants of thinner mothers benefited from early prenatal food supplementation in problem solving support and cover test scores whereas no such benefit was observed among infants of well nourished mothers (Figure 4.2. & 4.3.).

Table 4.15. Effect of nutritional interventions in low and high BMI mothers, on “problem solving total scores” and “problem solving intentional solutions” of their infants.

Developmental outcome	Variable used in the equation	Type III Sum of squares	df	Mean Square	F value	P value
Support total score (transformed) Adjusted R squared = .012	Age of testing	18.95	1	18.95	11.42	0.001
	Sex of child	8.38	1	8.38	5.05	0.03
	Food group	1.45	1	1.45	0.87	0.35
	Micronutrient group	4.73	2	2.37	1.43	0.24
	Breast feeding group	0.01	1	0.01	0.01	0.94
	BMI	12.27	1	12.27	7.39	0.007
	Food x MN group	3.13	2	1.564	.943	0.39
	BMI x Food	8.16	1	8.159	4.919	0.027
	BMI x Micronutrient	5.51	2	2.753	1.660	0.19
Cover total score (transformed) Adjusted R squared = .004	Age of testing	7.75	1	7.75	6.05	0.01
	Food group	0.14	1	0.14	0.11	0.74
	Micronutrient group	0.9	2	0.45	0.35	0.7
	Breast feeding group	1.4	1	1.4	1.09	0.3
	BMI	5.01	1	5.01	3.91	0.048
	Food x MN	0.28	2	0.14	0.11	0.9
	BMI x Food	5.09	1	5.09	3.97	0.047
	BMI x Micronutrient	2.52	2	1.26	0.99	0.37
Support Full+ Partial intentional solution Adjusted R squared = .011	Age of testing	14.1	1	14.95	6.5	0.011
	Sex	19.15	1	19.15	8.32	0.004
	Food group	3.73	1	3.73	1.62	0.2
	MN group	7.05	2	3.53	1.53	0.22
	Breast feeding group	0.41	1	0.41	0.18	0.67
	BMI	19.34	1	19.34	8.4	0.004
	Food x MN group	1.93	2	.96	0.42	0.66
	Food x BMI	12.29	1	12.29	5.34	0.021
	MN x BMI	3.28	2	1.64	0.71	0.49
Cover Full+ Partial intentional solution Adjusted R Squared = .001	Age of testing	1.82	1	1.82	1.07	0.3
	Food group	0.17	1	0.17	0.1	0.75
	Micronutrient group	1.2	2	0.6	0.35	0.7
	Breast feeding group	1.03	1	1.03	0.6	0.44
	BMI	4.7	1	4.7	2.76	0.1
	Food x MN group	1.33	2	0.67	0.39	0.67
	Food x BMI	6.74	1	6.74	3.96	0.047
	MN x BMI	5.4	2	2.7	1.58	0.21

4-way ANOVA with BMI as an additional factor and controlling for covariates

Figure 4.2. Effect of food supplementation of low and high BMI mothers on their infants' problem solving support test scores

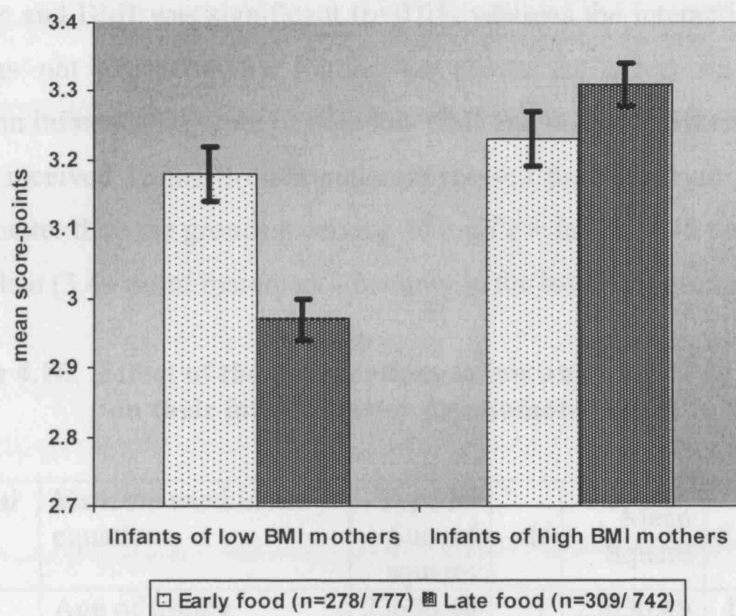
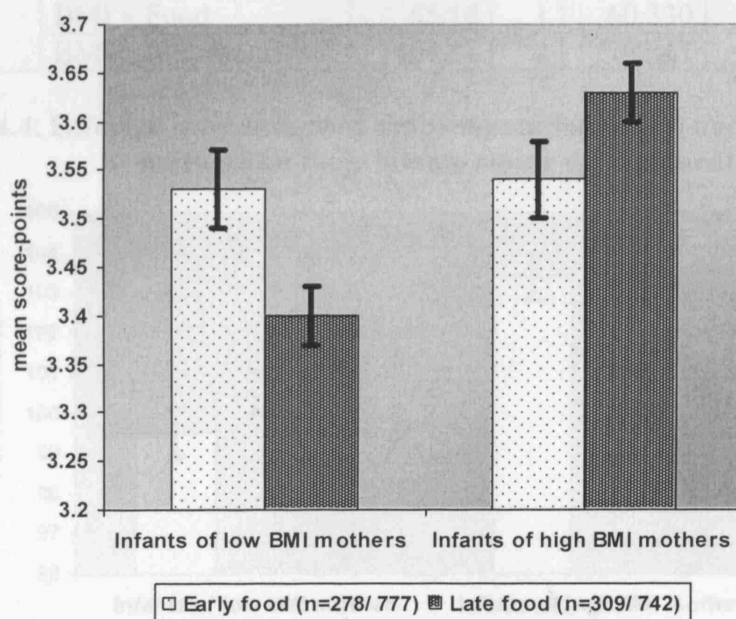


Figure 4.3. Effect of food supplementation of low and high BMI mothers on their infants' problem solving cover test score



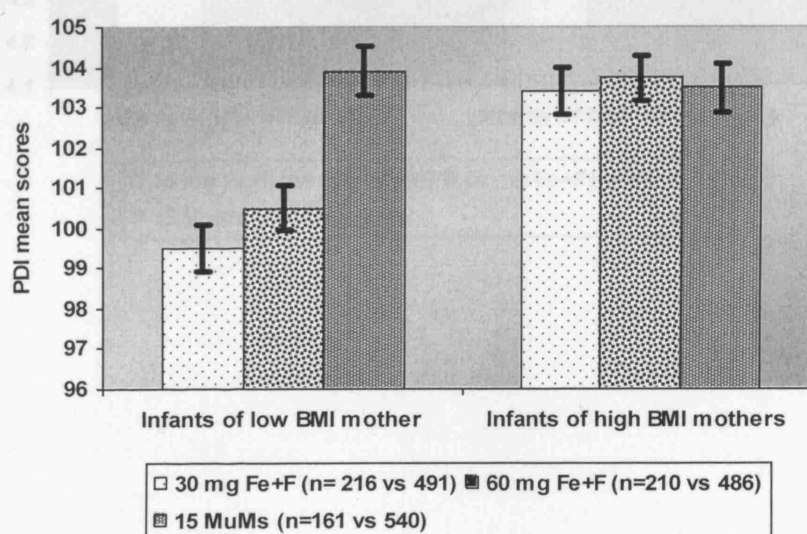
Psychomotor developmental index (PDI):

In the analyses of PDI there was significant independent effect of micronutrients ($p=0.023$) and BMI was also significant ($p=0.003$). The interaction between micronutrients and BMI was significant ($p=0.05$) whereas the interaction between food and BMI was not (Table 4.16). Figure 4.4 shows the effect of 3 micronutrient supplements on infants' PDI score of both low BMI and normal BMI mothers. Infants of mothers who received 15-multi-micronutrients showed the maximum benefit and were significantly better than the groups receiving 30 mg Fe + folate (4.40 point increment) or 60mg Fe + folate (3.41 point increment), but only in the low BMI group.

Table 4.16. Effect of the interventions in low and high BMI mothers on their infants Bayley psychomotor index

Developmental outcome	Variable used in the equation	Type III Sum of squares	df	Mean Square	F value	P value
Psychomotor Index (PDI) Adjusted R squared = 0.051	Age of testing	23630.49	1	23630.49	100.88	<0.001
	Food group	28.75	1	28.75	0.12	0.73
	Micronutrient group	1764.18	2	882.09	3.77	0.023
	Breast feeding group	2.74	1	2.74	.012	0.914
	BMI	2027.79	1	2027.79	8.66	0.003
	Food x MN	830.43	2	415.22	1.77	0.17
	BMI x Food	65.16	1	60.330	.270	0.6
	BMI x Micronutrient	1404.81	2	702.41	3.0	0.050

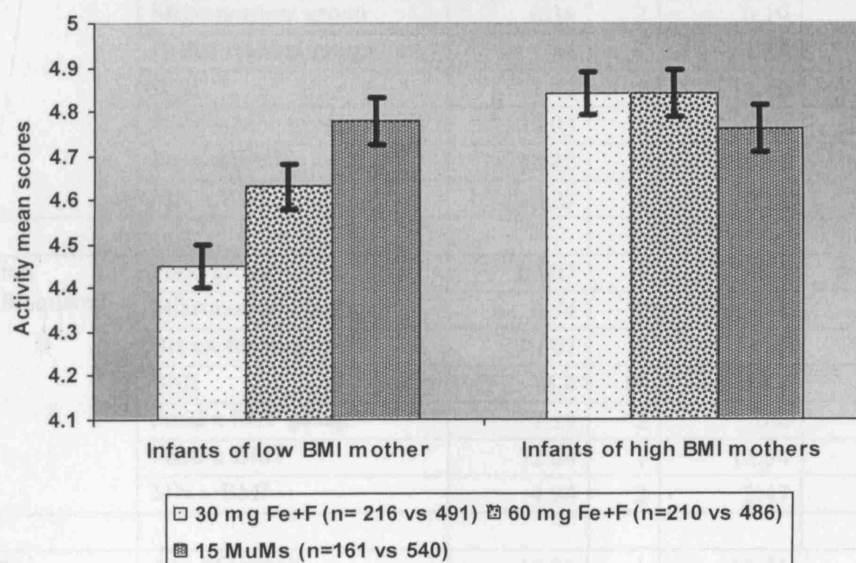
Figure 4.4. Effect of micronutrient supplementation of low and high BMI mothers on their infants motor development



Behaviour ratings during test:

We then examined for the effect of treatment on the behaviour ratings (Table 4.17.) of infants of low and high BMI mothers using similar ANOVAs. BMI (higher scores in infants of high BMI mothers compared to infants of low BMI mothers) was significant in emotion ($p=0.029$), vocalisation ($p=0.017$) and activity ($p=0.004$) ratings but not in approach or co-operation. The interaction between BMI and micronutrients was significant in the analysis of activity ($p=0.048$) and approached significant levels in analyses of emotion ($p=0.08$) and co-operation ratings ($p=0.07$). Figure 4.5. shows the interaction on activity, where the multiple-micronutrient supplementation in low BMI mothers significantly improved their infants' activity levels when compared to other two iron-folate supplemented groups. On the other hand, micronutrient-supplementation made no difference to activity levels of infants of normal BMI ($>18.5 \text{ kg/m}^2$) mothers.

Figure 4.5. Micronutrient effect on activity of infants of low and high BMI mothers



**Table 4.17. Effect of supplements in low and high BMI mothers
on the behaviour ratings of their infants**

Developmental outcome	Variable used in the equation	Sum of squares	df	Mean Square	F value	P value
Approach Adjusted R squared = -.002	Food group	2.93	1	2.93	2.03	0.16
	Micronutrient group	0.28	2	0.14	0.1	0.91
	Breast feeding group	0.19	1	0.19	0.13	0.72
	BMI	0.08	1	0.08	0.05	0.82
	Food x MN group	0.92	2	0.46	0.32	0.73
	Food x BMI	0.01	1	0.01	.007	0.93
	MN x BMI	1.85	2	0.93	0.64	0.53
Activity Adjusted R squared = .006	Sex of child	8.18	1	8.18	4.32	0.038
	Food group	0.61	1	0.61	0.32	0.57
	Micronutrient group	4.48	2	2.24	1.18	0.31
	Breast feeding group	0.76	1	0.76	0.4	0.53
	BMI	15.94	1	15.94	8.41	0.004
	Food x MN group	2.10	2	1.050	.554	0.57
	Food x BMI	1.2	1	1.195	.631	0.43
	MN x BMI	11.53	2	5.764	3.042	0.048
Emotion Adjusted R squared = .009	Sex	15.02	1	15.02	6.31	0.012
	Food group	0.009	1	0.009	.004	0.95
	Micronutrient group	0.38	2	0.19	0.08	0.92
	Breast feeding group	1.84	1	1.84	0.77	0.38
	BMI	11.42	1	11.42	4.8	0.029
	Food x MN group	10.03	2	5.01	2.106	0.12
	Food x BMI	10.42	1	10.42	4.379	0.037
	MN x BMI	12.23	2	6.12	2.570	0.08
Vocalization Adjusted R squared = .003	Food group	0.007	1	.007	.002	0.95
	Micronutrient group	0.74	2	0.4	0.13	0.87
	Breast feeding group	0.07	1	0.07	0.02	0.91
	BMI	16.4	1	16.4	5.67	0.018
	Food x MN group	7.19	2	3.6	1.24	0.30
	Food x BMI	12.64	1	12.64	4.37	0.038
	MN x BMI	4.94	2	2.47	0.85	0.43
Co-operation (transformed) Adjusted R squared = .012	Age of testing	16.31	1	16.31	6.84	0.009
	Sex of child	23.27	1	23.27	9.760	0.002
	Food group	0.49	1	0.49	0.21	0.65
	Micronutrient group	0.15	2	0.08	0.03	0.97
	Breast feeding group	1.18	1	1.18	0.5	0.48
	BMI	6.19	1	6.19	2.6	0.11
	Food x MN group	9.03	2	4.51	1.89	0.15
	Food x BMI	10.34	1	10.34	4.34	0.037
	MN x BMI	11.78	2	5.89	2.47	0.09

The interaction between BMI and food was significant in analyses of vocalization ($p=0.038$), emotion ($p=0.037$) and co-operation ($p=0.037$). Figures 4.6., 4.7. and 4.8. shows these interactions. Infants of mothers with low BMI benefited from early food. They significantly vocalised and co-operated more and remained happier during the test procedures compared to infants of low-BMI-mothers who were supplemented with late-food. No such benefit was observed in infants of mothers with high BMI with early food supplementation. In this group infants of mothers with late food supplementation showed better score on their behaviour compared to early food supplementation.

Figure 4.6. Food supplement effect on the vocalization rating of infants of low and high BMI mothers

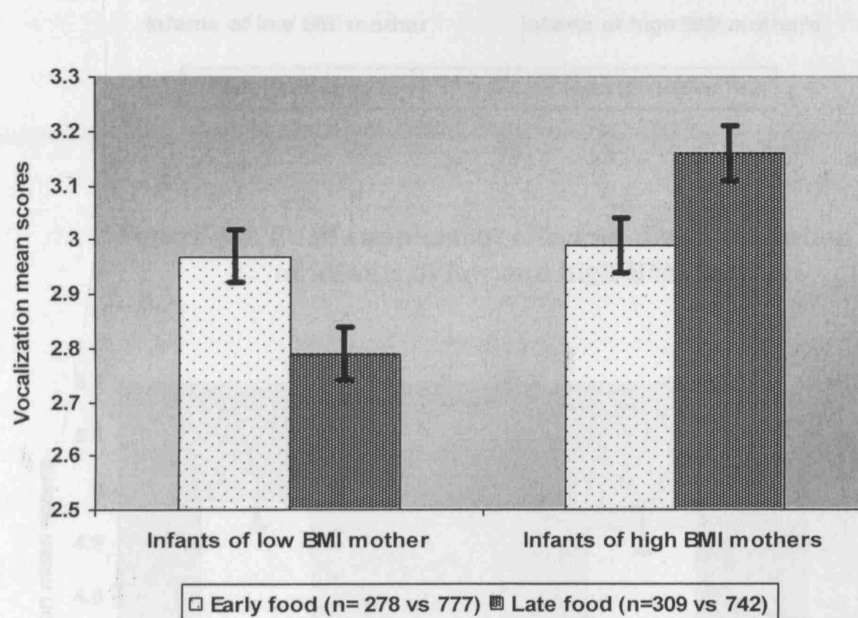


Figure 4.7. Food supplement effect on the emotion ratings of infants of low and high BMI mothers

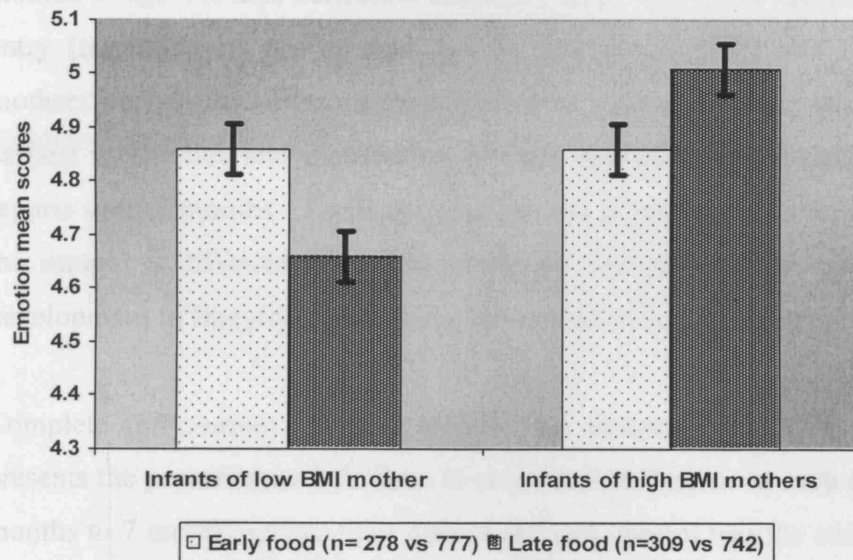
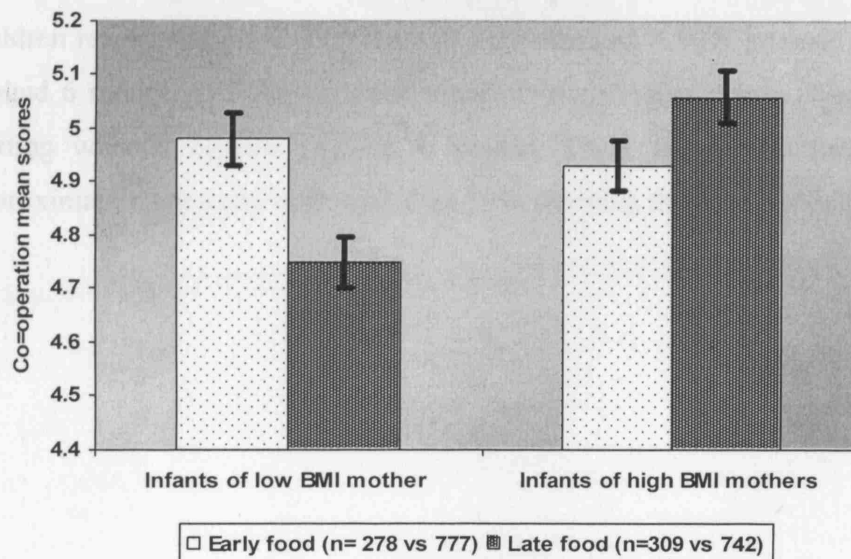


Figure 4.8. Food supplement effect on the co-operation ratings of infants of low and high BMI mothers



4.6 Milestone Reports:

Monthly information was collected on milestone attainment from 3 months until 12 months of age. As data collection continued there were some technical problems in data entry (testers-report not entered due to shortage of staff) and I could extract only mothers' reports on milestone attainment until 7 months. Here I shall present only the 6 earliest milestones and distribution of their age of attainment according to mothers' reports until 7 months. I will not examine the possible effects on intervention because the sample is different from that previously reported and the intent was to describe development in Bangladeshi children rather than to detect an intervention effect.

Complete information up to 7 months was extracted for 2376 infants. Figure 4.9. presents the percentage of children to attain each milestone at each monthly visit from 3 months to 7 months of age. The data show neck control was the earliest milestone to be attained and was attained by 78% of infants by 3 months. The next milestone to be attained was lifting chest in the prone position, and 22% attained this at 3 months and 73% at 4 months.

Sitting with support and picking up a cube were attained next with 83% and 65% of children respectively attaining them at 5 months and > 90% attained both milestones at around 6 months. Almost no child attained "transferring object from hand to hand" or "sitting without support" before 4 months. These two milestones were attained at approximately the same time with over 70% attaining them by 6 months.

Figure 4.9. Cumulative frequency of percent to attain certain milestones from <3-7 months of age.

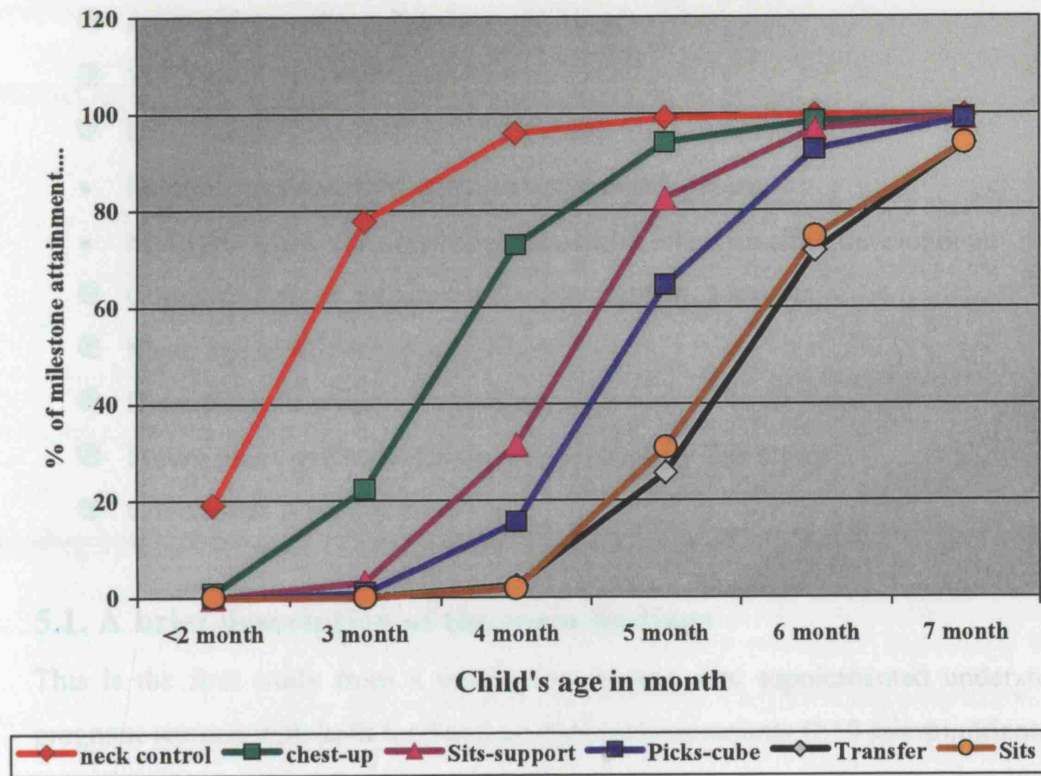


Table 4.18. Mean age (SD) of attainment of milestones

Milestones	Mean age in days of attainment \pm SD
Neck Control	93 \pm 27
Lifts chest -up	125 \pm 28
Sits with hand supports	147 \pm 27
Picks up a cube	159 \pm 25
Transfers object from hand to hand	183 \pm 29
Sits alone (unsupported)	184 \pm 28

Chapter 5: Discussion

This chapter contains the following sections:

- ⌚ A brief description of the main findings
- ⌚ Validity of the Treatment Trial
- ⌚ Discussion of the findings of the study
 - Food supplementation effect on child development
 - Multiple-micronutrients supplementation effect on child development
- ⌚ Comparing study findings with other published reports
- ⌚ Mean age of milestone attainment
- ⌚ Discussion on policy implications
- ⌚ Future plans and research questions raised by this study
- ⌚ Conclusion from the study

5.1. A brief description of the main findings

This is the first study from a developing country that supplemented under nourished pregnant women with both food and multiple micronutrients (>10 micronutrients) during pregnancy and observed their individual as well as an interactive effect on subsequent child development.

Our findings suggests that there is no overall benefit of prenatal supplementation with early food or multiple-micronutrients (UNICEF/UNU/WHO) compared late food or iron and folate supplementation on subsequent problem solving ability, motor development and behaviour of the infants when assessed at 7 months of age. However when we examined whether maternal malnutrition (BMI <18.5 kg/m²) modified the treatment effect according to the original hypothesis, a small but statistically significant benefit was found both from early food and multiple- micronutrient supplement. The children of malnourished mothers benefited whereas the children of better nourished mothers did not.

Early food supplementation benefited children of malnourished mothers in the problem solving tests, support and cover. Food also benefited their behaviour - they were less fussy, more happy and more cooperative with the test situation and vocalised more often than children of mothers given late food supplementation. Analogously, small but significant benefits on motor development and activity were observed among the infants of malnourished mothers who received multiple-micronutrient supplements.

The importance of mothers' BMI is demonstrated in the findings of a significant independent effect of BMI on all three tests - the support test, cover test and motor development. BMI also had a significant independent effect on three out of five behaviours with children of mothers with low BMI, being less active and vocal and more fussy during the test.

Our findings do not support the hypothesis 1 and 2 (chapter 2) where we expected neurodevelopmental benefit of infants of all pregnant mothers who received either early food or multiple micronutrients. However findings support our 3rd hypothesis where the infants of malnourished mothers benefited from early food and multiple micronutrients. There was no interaction between multiple micronutrients and early food on child development and the effects were additive, which supported our 4th hypothesis.

5.2. Validity of the Treatment Trial

I shall describe the adequacy of my research design as well as the possible threats to its validity under four levels of evaluations as described by Cook and Campbell (1979). They are - (i) statistical conclusion validity, (ii) construct validity of putative causes and effects, (iii) internal validity and (iv) external validity. As my study is a field based study, these assessments should adequately measure the validity of my trial.

5.2.1. Statistical conclusion validity

Statistical conclusion validity measures the extent to which the data allow the investigator to draw a conclusion with confidence about existence of a statistical relationship, in any direction. The threats to this type of validity concern low statistical

power of the study, violations of the assumptions of the statistical tests used (appropriate statistical tests), the use of multiple statistical tests, low reliability of the independent and/or dependent variables, sampling errors (random heterogeneity of subjects), and random irrelevancies in the experimental settings. I shall discuss these points in relation to my studies.

Sample size and power of the study

Insufficient statistical power in a study due to too small sample size can cause “Type II errors” i.e. false negative findings. In this study, 250 children in each group were sufficient to detect a difference of 5 developmental quotient (DQ) points for Bayley psychomotor test and 2.5 points for problem solving test among the groups with a power of 90% at 5% level of significance. In social science research, sample size considering a difference of half a standard deviation is used to calculate the power of the study as it is generally considered to represent a functionally significant effect. In our calculated sample size we are able to detect an even smaller difference among the groups. However we planned to follow up the sample into later childhood and ask other additional questions so when we found 2853 live-singleton babies born during the fixed time period (May 20, 2002 to December 20, 2003), we recruited them all and therefore had approximately 475 in each group.

Use of appropriate statistical tests

Appropriateness of statistical measurements is also an important factor that might influence statistical conclusion validity. We randomized subjects at individual level and grouped them into 6 supplement groups. Thus our experimental findings were analysed using 2 way analysis of variance (ANOVA) for intention to treat analysis with the two types of interventions (food and micronutrients) as factors controlling for possible covariates. For further analysis to examine the effect on malnourished mothers we conducted the same tests but used BMI (coded as low =1 and high=2) as an additional factor. All data were checked for normality and transformed where necessary. The assumptions of equality of variance and linear relationships between variables were met.

Multiple statistical tests

Use of a large number of statistical tests can cause an increased likelihood of falsely concluding that a statistical relationship exists when it does not. Thus for the main and subsequent analyses only one test (2-way ANOVA) is used, so reducing the possibility of “type I error”.

Measurement reliability or reliability of assessment

Lack of reliability of both independent and dependent variables used in the study can cause Type II errors by inflating the standard errors and increasing variability of the estimates. Three main types of random error can occur during measurements. These are observer variability, instrument variability and subject variability (Hulley et al, 2001) and can be minimized by standardizing the measurement methods and/or by training the testers and/or by refining the instruments. We used these strategies for all our dependent variables as described in details in “methods section”.

For all the psychological tests, initial and on-going inter-observer reliabilities were assessed and procedures are described in detail in the methods section. Test-retest reliability was assessed before the study began. The developmental tests were administered by four psychologists in the four subcentres. To minimize tester-bias we rotated them across the centres every third month. Subject variability is more difficult to control but we postponed tests where the child was very fussy due to tiredness or hunger. Intra class correlation coefficients (R) for each developmental test are given below.

(i) *Inter-observer reliabilities:* Test scores on 20 infants were compared between each tester and trainer. For PSTs the reliability was high ($R > 0.95$) for both support and cover tests, for Bayley motor $R = 0.95$ and for all behaviour ratings $R > 0.89$.

(ii) *Test-retest reliability:* Test-retest reliability over a short time interval (24 hour) for both support and cover PSTs on 10 infants was $R > 0.75$ and for Bayley motor test-retest reliability over a 7 days interval was $R = 0.9$.

(iii) *Ongoing reliability*: To maintain quality of testing ongoing interobserver reliability was assessed on approximately 7% of all milestone assessments, problem solving tests and Bayley tests where score of all the tests was observed by a supervisor. Intraclass correlations for all measures were - milestones $R > 0.86$, PST support test $R = 0.98$, PST cover test $R = 0.95$, Bayley motor $R = 0.97$ and for all behaviour ratings $R > 0.84$

Among the independent variables, anthropometric measurements and baseline information were collected by other well experienced investigators of the team with well maintained quality control. Research-assistants and health workers were responsible for measuring the children and mothers. Proper training was given to them until they attained satisfactory reliability. Digital scales were used to weigh the children to minimize observer error. For measurement of length, height and head-circumference standard procedures were used and inter-observer reliability was high ($R > 90$) for all anthropometric measures.

Baseline information was collected using interviewer administered questionnaires. These questionnaires were written clearly and instructions given about how the questions should be asked and the probes to be used where necessary.

In the main study, interviewers were trained for all above measurements until satisfactory reliability was achieved. Ongoing quality control was maintained for 5 to 10% of all the measurements to keep observer-variability to a minimum.

Sampling error

There is a possibility of sampling error due to heterogeneity of sample or lack of uniformity in administration of treatment or tests (Barrett, 1984). The population was reasonably homogenous, they were all living in a poor rural community with little variation in socio-economic backgrounds and parental literacy. Our main developmental measures, PST, PDI and five behavioural tests were administered in standardized test

environments at the sub-centres. The infants were tested at similar ages and the testers were well trained and the method of administration was standardized.

Distribution and consumption of nutritional supplements were very difficult to control for uniformity among all the subjects, even in a same treatment condition. In our study, we personally had no control over how supplements were administered or consumed. To ensure maximum intake, food supplements were distributed to each woman through the feeding centres and they were encouraged to eat them in the centre and aggressively discouraged from taking them home. However, it was anticipated that some women, especially in advanced stages of pregnancy would take packets home, but these records were also kept. Compliance on food supplementation consumed by mothers was well monitored throughout the study (described in methods section). Although compliance was monitored using a number of different sources of information (section 4.3). Even after all these measures there remained considerable variation in the use of supplements which might interfere with the statistical power of the test by inflating the error variance and increasing the chance of Type II error. Controlling for compliance did not change the findings for our main study, thus indicating that variation in treatment did not affect the statistical conclusion validity of our finding. However, as the final compliance report considering all information is not available for this analysis, it might be a threat to the validity of our findings.

Random irrelevancies in the experimental settings

This threat includes features in experimental settings other than treatment that might affect the scores of the dependent variable to inflate the error variance (Cook & Cambell, 1979). This is difficult to control in complex field settings. However anticipation and measurement of such extraneous variance, which are common to all treatment groups and controlling them in the statistical analysis if they are valid can minimize this error. We tried to collect all possible information about factors other than treatment that might influence the outcome variable eg, parental education, socio-demographic variables e.g. maternal biological measures, age and sex of child etc and

controlled for them in the analysis if they differed among groups and correlated with outcome.

Considering the above, it can be concluded that we have reasonably satisfied the conditions for statistical conclusion validity and thus can confidently report our empirical finding.

5.2.2. Internal Validity

Internal validity refers to the degree of confidence with which we can conclude whether there is any causal relationship between the dependent and independent variables (Cook & Campbell, 1979).

Internal validity is weakened when there remain alternative explanations for the observed effect and is strengthened when randomisation to treatment group is done with appropriate control groups (Barrett, 1984). Probably the most important point in determining internal validity is randomization and the present study was a RCT. In this case the groups may be considered equivalent in all possible confounders and only vary in treatment and it is possible to make causal inferences assuming other threats to validity are not present. Other threats to internal validity include:

“History” and maturation: These threats refer to confounding events (poor educational history, poor health, impoverished physical environment etc) occurring between the administration of treatment and measurement of the dependent variables. According to Jensen (1974, 1977), a special case of maturation history is “cumulative deficit” that refers to a process by which the children from under privileged populations perform poorer in cognitive tests overtime compared to children of non-under privileged populations. Our population was a homogeneous one in terms of social and economic background and of a similar age. Further, this threat is minimized due to randomization of the population into different treatment groups.

Mortality: A total of 121 infants died in our study and constitute 16% of total loss. The number of deaths was equally distributed among the intervention groups and thus there is little possibility of mortality being a confounding factor.

Familiarity with a test: It was not a threat for our study as all infants were exposed to the developmental tests in a controlled environment for the first time. Though they were tested for motor milestones every month at home, it was based on two to three new motor skills the child attained and not comparable to the series of gross and fine motor activities assessed by the PDI of the Bayley scale.

Instrumentation: It is important to use the same instruments and testers for all groups. It was not a problem as the same standard tests by the same testers were applied to all the children once at a fixed age range of around 7 months.

Statistical regression to the mean effects: It is not relevant to our study as different treatment groups in our study are not selected on the basis of any baseline score. Rather they were selected randomly from a population at around 8-10 weeks of pregnancy.

Ambiguous direction of causality: It is particularly applicable for observational studies and not applicable for our RCT and treatment preceded the assessment of development.

Diffusion or imitation of treatment: This means when a particular group which is not assigned to treatment receives treatment informally either by sharing the treatment with the supplemented group or by eliciting similar treatment from the investigators.

In our double blind randomized control trial it was not a problem for micronutrient supplementation, as all the groups received pills that either contain multiple micronutrients or iron-folate. However food supplementation was not double blind. Mothers randomised to early food supplementation were encouraged to start early at around the tenth week of pregnancy. Whereas, the late supplemented group could make their own choice of when to start. This may have caused some overlapping between the

early and late group at the start of supplementation. However, previous experience from a similar study showed that if mothers were not encouraged/ persuaded to take early supplementation, they preferred to start at around the 17th week of gestation (Shaheen et al, 2000). Thus a little overlapping could have occurred in our study (~10 % personal communication Shams El Arifeen) but it is unlikely to be a serious threat to our internal validity.

Compensatory equalization – This happens when the researcher abandons the planned treatment and gives all the groups the most beneficial treatment therefore none is deprived. This was not a problem in our study.

Compensatory rivalry - Here the subject, who receives less favourable treatment, tries harder to perform better in assessment or measures compared to the benefited group. As mothers all received some supplementation and infants were tested at 7 months of age, this is highly unlikely to be a problem.

Resentful demoralization – In contrast to compensatory rivalry, here the less advantaged group tries less hard when assessed for treatment effect and is therefore not applicable to our study.

Thus our study seems to have a sound internal validity with minimum threats.

5.2.3. External Validity

External validity refers to the extent to which a research finding can be generalized across different samples, population groups, experimental conditions and settings (Cook & Campbell, 1979). In population based nutritional research the issue of generalizability is very important. However, extrapolation of studies on nutritional intervention in different populations is very difficult as the prevalent nutritional deficiencies may vary. The effect of nutrition on child developmental measures may further vary as it may be modified by the quality of the home environment (Walker et al, 2004).

Random sampling from the total population is the best possible way to be able to extrapolate the findings to the whole target population. Usually replication of studies in different setting is also done to test external validity.

Our sample included all available pregnant women from a rural community irrespective of their nutritional condition. The study area was slightly better than some of the remote rural areas of the country in terms of socio-economic background and living standards because of being situated nearer to the capital and the availability of the ICDDR,B clinic services. However, the prevalence of LBW (28%), stunting and wasting in under-five children is respectively 68-76% and 15%-30%, and mean maternal BMI 20.1 kg/m², thus they were similar to the national average reported recently (Table 1.20). We can therefore be reasonably confident that the results can be generalized to undernourished populations in rural Bangladesh. However, the effects of micronutrients in the present study occurred in women who also received food so these results should only be extrapolated to Bangladeshi populations who receive similar food supplementation. Before extrapolating to populations in other developing countries it would be advisable to replicate these findings in small controlled studies. Differences in nutritional deficiencies and current diet could modify the effects.

5.2.4. Construct validity of putative causes and effects

“Construct validity of putative causes and effects indicates the degree to which our conceptualizations of dependent and independent variables match the operationalization of the measurements (and therefore the extent to which the theoretical inferences we draw from the findings are empirically justified” (Barrett, 1984). Practically, it addresses the question of conceptual validity of the research findings. It also explains the validity of interpretations as well as the inferences drawn from study finding. According to Cook & Campbell (1979), threats to construct validity include:

Inadequate pre-operational explanation of concept: This includes proper analysis of the theoretical concept of the study and adequate conceptualization of the dependent and independent variables, leading to appropriate operationalizations of the variables.

Thus there are concerns with the theoretical explanation of how the independent variable influences the dependent one. In the present study we hypothesized that early food and multiple micronutrient supplementation during pregnancy would be beneficial for the development of the offspring.

The explanation behind the concept of early food supplementation highlights its effect on brain development. We hypothesized that early maternal nutrition would supply adequate nutrition to the developing brain via the placenta because brain development starts very early at around 8-10 weeks of pregnancy (Johnson, 2005). We also hypothesized that the supply of additional early food to these poor undernourished mothers would improve the subsequent development of infants via lowering the incidence of LBW infants (Bhatia et al, 1979). This would minimise the adverse effect of LBW on brain weight, brain lipids (particularly myelin lipids) and cellularity (Chase et al, 1972) thus improving cognitive and behavioural outcome of infants (section 1.4.).

The other reasons why early food supplementation might be beneficial include the following. First, it has been observed that most food-programmes start (eg BINP) supplementating in the middle-trimester. This happens partly because the women do not seek prenatal care until the second trimester and partly due to the biological concept that food supplementation exerts the greatest benefit on growth during the last trimester of pregnancy. There is evidence that growth retardation in foetuses can occur early between 8-18 weeks of gestation (Smith *et al.*, 1998; Neufeld *et al.*, 1999). Second, epidemiological studies have also shown an association of maternal malnutrition during early pregnancy with adverse birth outcomes i.e. newborns were short, light, and had small head circumferences (Thame et al, 1997; Arbuckle & Sherman, 1989). Third, according to Li and colleagues (1998), a one kg increment in maternal weight in the second trimester can cause double the effect on birth weight than one kilogram increment in weight in the third trimester. Fourth, there are reports that thinner mothers can have lower placental/birth weight ratio due to some insult in the placenta (Thame et al, 1997), which very likely occurs in the first half of gestation (Owens et al, 1995).

In the present study a strong association between maternal BMI at enrolment and subsequent development of the offspring emphasises the importance of early nutrition.

The explanation for mechanism of multiple-micronutrient supplementation during pregnancy on brain development of offspring is less clear (section 1.4.2.). However, some individual micronutrients (components of the multiple-micronutrient mixture) have been reported to exert a direct as well as indirect (through foetal growth) effect on foetal brain development. I could locate evidence for an individual role of thiamine (vitamin B1), pyridoxine (B6), zinc and iodine excluding iron and folate which was given to the comparison group in my study on brain development (details of mechanism are described in section 5.3.2.), mostly in animal models. Some micronutrients like niacin, vitamin B12 and copper have been reported to have an effect on the nervous system in humans (vitamin B12, vitamin E and niacin) but their role during pregnancy on foetal brain is not clear. However, deficiency of some of them have been reported to cause foetal growth restriction eg niacin and vitamin E (Institute of Medicine, 1990).

We used two different tasks to assess problem solving. The basis of the tasks was first described by Piaget and the conduct and scoring of the tests were designed by Willatts (1999, 1984). The tests include simple one-step means to manipulation of an intermediary object (a cloth) to retrieve a goal (toy). The tests measure mainly the ability to solve a problem, which also requires some memory and attention as the child has to pay attention to the task and remember that the toy is hidden. The behaviour ratings were originally based on Bayley ratings but had been modified by Wolke to facilitate attaining good inter-observer reliability (for all behaviour ratings $R > 0.89$). The Bayley motor scale assesses a comprehensive range of gross and fine motor skills. In the development of this scale the items were subjected to scrutiny by a panel of experts who concurred that the major domains of motor development were covered by the test. None of the measures used has been standardized for use in Bangladesh.

However all have been reported to pick-up differences due to nutritional interventions in other study countries (Gardner et al, 2003; Willatts et al, 1998; Hamadani et al, 2001; 2002; Jahari et al 2000). The justification for using these tests has been described in detail in the methods section (Chapter 3). The tests were used to make comparisons among the different intervention groups and no attempt was made to interpret the absolute level of the scores or compare these scores with other populations. The tests were pre-tested in this population for cultural appropriateness.

Mono-operational biases: This occurs when only one aspect of the dependent variable is measured by the investigators or operationalization of independent variables was done in a restricted way, for example, if only verbal ability is assessed to measure the cognitive development of the malnourished infants who were supplemented with food (Barrett, 1984). We assessed three different aspects of development: behaviour, motor development and cognition. However, we measured a fairly limited sample of cognitive functions and problem solving ability. The narrow range of cognition measured might be a threat to the validity of the findings. It is possible that changes occurred in unmeasured domains of development.

Mono-method biases: This occurs when only one “form” of assessment is considered to study the dependent variable while examining the relationship between dependent and independent variable (Barrett, 1984). For example, if only the child’s response to a specific structured testing situation is considered but not behaviour in normal situations. In a large field trial it is difficult to assess behaviour in normal situations. However we did have behavioural ratings as well as structured tests. It remains possible that more ecologically valid measures such as behaviour at home may have been affected by supplementation.

Hypothesis guessing and evaluation apprehension: Subject bias is introduced when the subjects guess the hypothesis and behave as they think they are expected to and/or when they try to portray themselves in the most favourable manner. This bias is probably not a threat to our outcome of the study as the children were too young to be affected by this.

Experimenter expectancies: Observer bias is introduced when the observer expects the experimental group to perform more favourably on a test or questionnaire. In this study the testers and interviewers were blind to the treatment status of the children and hence this bias was not present.

The confounding of construct with level of construct: It refers to generalization that is made when only one level of treatment is measured. In our study it was hypothesized that early food supplementation during pregnancy compared to late supplementation and multiple micronutrient supplementation compared to only iron-folate supplementation which would benefit the subsequent developmental outcome of the children. Early food supplementation was started soon after diagnosis of pregnancy around the 8th -10th week of pregnancy whereas late food supplementation began around the 17th week of pregnancy. However there was a 10% overlap between the groups at the time of starting the food supplements. It would have been more useful if there was one group without any extra food or micronutrient supplementation. However this was not ethically possible because the community was already receiving food and iron-folate as part of government policy.

The interaction between different treatments and between treatments and prior testing: Another threat to construct validity of putative causes and effects occurs when subjects receive more than one treatment as the effect may be due to the combination of treatments rather than to one of the treatments alone. The present study was designed to examine any interaction between the late food and multiple micronutrients and there were no interactions between the treatments. However as all mothers had some food the effects may only be valid for mothers receiving some food supplements. Importantly, we cannot isolate the effect of the late food.

Therefore though most of the criteria of construct validity were fulfilled, some weaknesses regarding mono-method biases and confounding of construct with level of construct remained.

5.3. Discussion of the finding of the study

5.3.1. Food supplementation effect on child development

Intention to treat analysis: Growth restriction can occur very early during pregnancy, around the 1st trimester (Smith et al, 1998; Neufield et al 1999), and intra uterine growth restriction is associated with adverse developmental outcome (Grantham McGregor, 1998; Hack, 1998). Pregnant women in Matlab are poor and have less than optimum dietary intakes (Kramer et al, 1997), particularly in energy, and have poor nutritional status (Alam et al, 2003). We had therefore hypothesized that infants born in the early supplemented group would be heavier at birth (Kramer & Kakuma, 2003) compared with the group supplemented later and this improvement would subsequently benefit the offsprings' development. However, not only was birth weight not affected but we failed to find an overall significant benefit on the infants' problem solving abilities, motor development and behaviour.

The targeted population was very poor with a mean BMI of 20 kg/m² and 28% of them were malnourished (BMI <18 kg/m²). Our other hypothesis was that infants of malnourished mothers would have the most developmental benefit. Children born to mothers with a low BMI showed significant benefits from early food supplementation in contrast to infants of better nourished mothers who did not benefit. The infants of undernourished mothers benefited in both problem solving tests but not in motor development. Infants of supplemented low-BMI mothers also benefited in behaviour, being happier, more cooperative and more vocal than similar infants of late-supplemented low-BMI mothers.

There are several possible explanations as to why there were no differences between early and late food-supplemented groups when all mothers are considered irrespective of their nutritional status. Firstly, and most importantly, there was no group without any food supplementation so the intended difference between the groups was small. The early supplemented groups were repeatedly asked to join the feeding programme as soon after their enrolment as possible, whereas the late supplemented group was left to make their own choice as to when to start supplementation. However, there was a slight

overlap (10%) in the time of starting the feeding programme between the groups, which would have further diluted the difference between them. Adherence to food supplementation was only moderate, around 1690 (60%) of the mothers had at least 50% of total assigned number of packets in this period and their uptake was well monitored. Rigorous information was collected on the reliability of the compliance measures. Ten percent of all three 24 hr recalls were repeated, and 10% of the monthly questionnaires given during home visits on food frequency and consumption of food packets were repeated. . Records were also kept of whether the food was eaten at the clinic they are not presently available so we do not know how many packets were taken home and shared. Also we do not know whether they substituted their usual diet. Unfortunately these data are presently not available to us. So we cannot comment on the reliability of monitoring of compliance. Secondly, although the nutritional status of the mothers was generally poor there was a variation. About two-thirds of the mothers had BMIs above the vulnerable cut off (18.5 kg/m^2) of malnutrition. Though they were from poor socioeconomic backgrounds, they were probably capable of fulfilling the early nutritional demands of the developing foetus. Later on the increased requirement of food by the growing foetus would have been met by both the early and late supplementation programme. It is therefore likely that in the better nourished mothers the foetus did not experience a nutritional crisis that would have affected the developing brain. This interpretation was further strengthened by results from the main study where early food supplementation showed neither increase in birth-weight nor reduction in incidence of LBW (personal communication). In our subsample there was also no difference between the groups in birth weight.

Analysis with low BMI mothers:

Infants of malnourished mothers in our study were significantly smaller in all birth measures (table 5.1) compared to infants of well nourished mothers, which suggests that the foetus was more vulnerable to nutritional deficiency. This is probably the reason why only the infants of low BMI mothers benefited from early food supplementation. However, early food had no effect on birth size including head circumference in low BMI mothers (personal communication, MINIMat team). So brain weight is unlikely to

be larger in the early supplemented group to any extent as there is a correlation between head size and brain weight (Ivanovic et al, 2004; Botting et al, 1998). However, other more subtle changes could have occurred such as altered functions of neurotransmitters, dendritic branching, connections and myelination (Johnson 2005; Chase et al, 1972).

Table 5.1. Birth measures in malnourished and well nourished mothers.

Birth measures	Infants of low BMI ($<18.5\text{kg/m}^2$) mothers (n=753)	Infants of high BMI ($>18.5\text{kg/m}^2$) mothers (n=1848)	P- value
Birth weight (mean \pm sd)	2595.32 \pm 399	2725.46 \pm 416	<0.001
Birth length (mean \pm sd)	47.42 \pm 2.3	47.92 \pm 2.3	<0.001
Head circumference (mean \pm sd)	32.06 \pm 1.8	32.58 \pm 1.7	<0.001
Ponderal index (mean \pm sd)	2.43 \pm 0.3	2.47 \pm 0.3	<0.001
LBW %	39.2 %	27.7%	<0.001

There is a suggestion that the nervous system is spared relative to the rest of the body (brain-sparing) during nutritional deprivation (Ballabriga, 1990, Dodge et al, 1975; Reinis et al, 1980). However, it is still unknown to what extent the brain is protected in malnourished mothers. I could locate only one very early small study that selected 25 severely malnourished pregnant mothers and compared their infants' behaviour with infants of 23 well nourished mothers (Bhatia et al 1979). The infants of severely malnourished mothers had gross growth retardation at birth and on neurological examination had significantly lower muscle tone and poorer reflexes than the other group. However they were not assessed for subsequent development.

5.3.2. Multiple-micronutrients supplementation effect on child development

In the present study, multiple-micronutrients (UNICEF/UNO/WHO) given to pregnant women did not benefit their children's problem-solving ability, behaviour or motor development at 7 months of age compared to children whose mothers received high or low dose iron-folate combinations. However, the interaction between BMI and receiving multiple-micronutrients was significant for the psychomotor developmental index (PDI)

and activity scores of infants in favour of multiple-micronutrient supplementation. Infants of malnourished mothers had better motor development and were more active when they received multiple-micronutrient supplements compared with infants of malnourished mothers who received iron and folate only. In contrast the infants of better nourished mothers did not benefit from multiple-micronutrients. The findings suggest small benefits to infants' motor development and activity levels from multiple-micronutrient supplementation but only when it is given to vulnerable malnourished women. These improvements occurred even though the compliance with taking micronutrients was only moderate; more than 60% of mothers had at least 50% of assigned tablets.

In our study multiple-micronutrient supplementation had no effect on birth weight or the incidence of LBW. Due to ethical reasons none of these pregnant mothers was without any micronutrient supplementation, so it is not possible to determine whether iron and folate benefited the infants. However there was no difference in infant development between the two iron doses.

We had hypothesised that multiple-micronutrients would benefit the total sample because it had been reported that pregnant women in developing countries usually suffer from co-existing deficiencies of several micronutrients (de Onis et al, 1998, Rao et al, 2001). Even after the implementation of a government programme of iron-folate supplementation for pregnant women, the prevalence of anaemia remained high at about 23% (Stoltzfus et al, 1998). It was thus hypothesized that there would be co-existing deficiencies of several micronutrients in this population and that this would affect the development of their offspring.

There might be a number of different causes for not getting any difference between the multiple-micronutrient supplement group compared to the two doses of iron-folate groups on birth measures as well as developmental measures in the total sample. Firstly, none of our supplement groups was without micronutrients thus we have no true control group. Secondly, interactions among the micronutrients in the multiple-micronutrients

group could limit their effect (Lind et al, 2004). Thirdly, it may be that the average population was not deficient enough in micronutrients (as 2/3rd were well nourished) to cause adverse effects on birth or development of the offspring. Fourthly, supplementing mothers with iron-folate in the comparison groups would have concealed any benefits from iron and folate. It is also possible that iron and folate reduced the effect of the other micronutrients due to unrecognised interactions.

Low BMI mothers:

We also hypothesized that these poor under-nourished (BMI <18.5 kg/m²) Bangladeshi mothers would be particularly deficient in micronutrients, which in turn could act as growth limiting factors for the growing foetus. Though recently a few studies have been conducted to address the role of prenatal-micronutrient use on birth-outcome (Osrin et al, 2005; Friis et al 2004; Christian et al, 2003; Ramakrishnan et al, 2003), I could not find any reports on its effect (>10 micronutrients) on brain development of the offspring and our study appears to be the first study addressing this question.

5.3.3. Effect Size

The findings indicate both early food and multiple-micronutrient supplementation in pregnancy in malnourished mothers benefit child development. However the effect size was very small and the confidence limit of the estimate was relatively wide. The critical question is whether the effect is functionally or clinically important and whether it is sustainable. For early food, the effect size on support test scores was $B \pm se$ 0.28 ± 0.1 CI 0.03 to 0.52 (p value). This is equivalent to 0.24 of a standard deviation score. The early food effect size on cover test was $B \pm se$ 0.22 ± 0.1 , CI 0.03 to 0.44 (p value). This is equivalent to 0.19 of a standard deviation score. For multiple micronutrients, the effect size on PDI was $B \pm se$ 4.27 ± 1.9 , CI 7.9 to 0.62 (p value), which is equivalent to 0.28 of a standard deviation score.

As we cannot be sure of the long term predictive value of these tests in this population the clinical and public health importance of this difference can only be determined in future follow-up.

5.4. Comparing study findings with other published reports

Food supplementation effect on birth weight and child development

In contrast to findings from several other studies we failed to find a benefit on birth size from early food supplementation during pregnancy (Kramer & Kakuma, 2003). Although unlike the other studies, our investigation was limited to the difference between early and late supplementation.

However our findings showed a benefit of food (energy and protein) supplementation during early pregnancy (8-10 week of pregnancy) on the infants' problem solving ability and some behaviour but only in infants of malnourished mothers. The only study I located that supplemented pregnant mothers and found benefits in child development was from Taiwan (Joos et al, 1983). In that study, mothers received supplementation throughout pregnancy from before conception and through lactation. The offspring showed benefits in motor development at the age of 8 months but not in cognition. The population was at least as undernourished as ours with a mean maternal BMI of 20.7 kg/m² at the beginning of the 3rd trimester compared with a mean BMI of 20.1 kg/m² at beginning of 2nd trimester in the present study (Data on BMI at 3rd trimester in our study is not available). The Taiwanese mothers also had a low protein intake (< 40 g/day), but data on protein intake of mothers of our study were not available yet. The mean weight gain from conception to delivery in Taiwanese mothers was 7.51 kg whereas in our study, mean pregnancy weight gain was around 5.5 kg during 8-30 wk of pregnancy (personal communication, MINIMat team). In Taiwan the supplementation was more intense and started soon after birth of the previous child and continued until delivery of the study child. The calorie content of the supplement was also higher than ours (800kcal vs 600kcal) with an added 40 g protein. Moreover, all the pregnant mothers in Taiwan received 22 micronutrients and continued supplementation throughout the lactation period. In our supplement no animal protein was included; a small amount of vegetable protein was within the food mixture and provided 25% (150 calories) of the total 600 calories. Although only half of the mothers in the early food

group received multiple micronutrients there was no difference in response to early food according to whether they received multiple micronutrients or not.

The reason why in Bangladesh the food supplement did not benefit the whole population whereas in Taiwan it did, is probably due to the fact that the difference between the early and late food supplementation in Bangladesh was much smaller than the difference between supplemented and placebo groups in Taiwan.

It is difficult to explain why the Taiwanese infants benefited in motor but not mental development from food whereas the Bangladeshi infants (of malnourished mothers) benefited in problem solving but not motor development. Both studies used the Bayley motor test to assess motor development whereas the Bayley mental test was used in Taiwan and the problem solving test was used in Bangladesh to assess cognition. I would hypothesize that the effect on motor was less in Bangladesh reflecting the small differences between the early and late fed groups. However it is possible that the problem solving test is more sensitive to subtle changes than the Bayley mental scale and that using the Bayley failed to detect benefits that occurred in cognition in Taiwan.

Three other pregnancy-trials with food supplementation were from developed countries (Pencharz et al, 1983; Osofsky, 1975; Rush et al, 1980) and showed no clear association of maternal prenatal supplementation and subsequent child development.

The New York study (Rush et al, 1980) showed increased preterm delivery, higher LBW and more neonatal deaths in the group that supplemented with high protein. However they also showed some developmental benefit in visual habituation in groups that received high doses of protein (40g/d), which the author claimed was not mediated through growth. It is interesting to note that the same amount (40g/day) of protein supplement which showed a beneficial effect in Taiwan (Joos et al, 1983) caused a harmful effect in a developed country (Rush et al, 1980). This suggests that the usual diet contained a much higher level of protein in New York than in Taiwan and illustrates

the dangers of extrapolating results of supplementation from one population to another with different characteristics.

Among the studies (detailed in section 1.5) that supplemented both mothers and infants (Chavez et al, 1975; Pollitt et al, 1993; Lechtig et al, 1975; Waber et al, 1981; Hicks et al, 1982) all showed consistent results with some developmental benefit to the children. However the effect of supplementation during pregnancy alone could not be assessed. In the Bogota study (Waber et al, 1981), one group of pregnant mothers received supplementation from the 3rd trimester of pregnancy until the 1st 6 months after delivery. Infants of these mothers showed small early developmental benefits but none remained at 3 years of age.

It is extremely surprising that there remains only one study (Joos et al, 1983) from a developing country that looked at the effect of food supplementation throughout pregnancy in poorly nourished women on the development of their infants. Unfortunately my study was limited to examine the effect of early versus late supplementation which reduces the chances of finding any benefit. There is an urgent need to determine the role of supplementation throughout pregnancy in malnourished women on infants' development in other populations.

Furthermore, my study appears to be the only one that looked at the effect of supplementation (food and micronutrients) in a specific time period of pregnancy on subsequent child development. This was a health services question and the main reason for this design was to provide information for policy makers regarding the effect of extending the duration of supplementation into the first trimester.

Multiple-micronutrient supplementation effect on birth weight and child development

As discussed in the literature review there is no consistent evidence that iron, iron-folate or zinc benefits birth weight. Research on the effect of supplementing with a wide range of micronutrients and vitamins combined during pregnancy are limited. I located 5 supplementation trials with apparently healthy women (Osrin et al, 2005; Friis et al

2004; Christian et al, 2003; Ramakrishnan et al, 2003; Hinninger et al, 2004) and all showed a consistent increment in birth weight with multi-micronutrient supplementation, though the improvement was generally small and not always significant. Among these, the Nepalese study (Osrin et al, 2005) found the greatest benefit in birth weight of infants from multiple-micronutrient supplementation compared to iron-folate.

I failed to find any study that supplemented pregnant mothers with multiple-micronutrients (>10 micronutrients) during pregnancy and observed subsequent infant development. Only one pregnancy trial (Dobo et al, 1998; Czeizel 1994) supplemented pregnant women during the periconception period with 16 multi-micronutrients and found no benefit of infants' DQ at the age of 2 years and IQ at the age of 6 years compared with a group receiving trace elements only. But significantly lower anxiety and tension among infants in the multiple-micronutrient supplemented group was found. Our study found improvements in motor development and activity score of infants in the multi micronutrient supplemented group but in malnourished mothers only.

A supplementation trial of infants (6-12 months) with multiple-micronutrients (2 RDA of 15 micronutrients) in the same community of Matlab also found improvements in motor scores on the Bayley compared to the placebo (riboflavin) group (Black et al, 2004a). However the doses of double RDA micro-nutrients were given directly to the infants and infants had more side effects like vomiting with multiple-micronutrient supplement. The infants' development in the multiple-micronutrient group was not significantly different from the group receiving only iron and zinc.

5.5. Mean age of milestone attainment

We only reported the distribution of attainment of each milestone based on the maternal report. Though the presented milestone data are based on maternal information, all mothers were well instructed about how to assess their child for each milestone and how to keep a record of the attainment date in the structured milestone calendar supplied to them. However, due to technical difficulties with data-entry, we were not able to extract

the testers' observation report on milestone assessment, so I limited this report to showing distribution of milestone attainments.

It was possible to compare the age of attainment of sitting without support with findings from other studies as several of them have used similar definitions of this milestone. The mean age of attainment in the present study was similar to that reported in an early study in Jamaica (Grantham-McGregor, 1972). It was slightly earlier than that found in a recent Nepalese study (Siegel et al. 2005) and considerably earlier than an older Indian study (Vazir et al, 1998). The attainment age was similar to the age of the US sample used to standardise the Bayley Scales (Siegel et al, 2005). It is surprising that these disadvantaged Bangladeshi children with a high incidence of LBW should attain sitting at the same age as the US children. However, the scores of the Bangladeshi children on the Bayley PDI provide a good measure of concurrent validity for the milestone measures. It is possible that good breast feeding and the lack of restriction in the children's environment account for their good development at this age.

5.6. Costs of intervention

The cost of the food supplement was 10 takas per packet, so if they received 6 packets a week for a full course from the 10th week of pregnancy to delivery the cost would be 1,800 takas (US\$30). On the other hand, the difference between early and late supplementation was only 30 packets which would cost 300 takas (US \$5). The cost of a multiple micronutrient tablet was 0.75 takas so if they took a full course of daily tablets from the 14th week of pregnancy to delivery, the cost would be 136.5 takas (US \$ 2.3). We do not know the cost of delivering the food or tablets. These costs have to be weighed against the benefits. We only know the benefits of providing early food compared with late. This benefit was small but could be important. However only long time follow-up will indicate the true benefit. More detailed assessment of cost-benefits is being done by other investigators.

5.7. Discussion on policy implications

It is important to monitor any prenatal intervention for its later consequences, not only on intrauterine growth but also on the later development of the offspring as the underlying mechanism of “foetal growth” is not simple (Gluckman et al, 1997). For policy implications it is also important to know who benefits, which time period of food supplementation has the maximum benefit, whether multiple-micronutrients benefit more compared to iron-folate and also whether food and multiple-micronutrients together exert the best effect in terms of foetal outcome. All these questions we tried to address in my study.

If multiple-micronutrient supplementation was found to be effective, then only a little modification would be needed to existing policy and programmes to bring benefits to a large number of women. The existing supplementation programme includes food supplementation for low BMI mothers whenever they prefer to start it during pregnancy (BINP, NNP, in Bangladesh) and 60 mg iron and 400 µg folate for all pregnant mothers worldwide (WHO, 2001). However before making firm recommendations for change more information is required including whether benefits are sustained, and whether other health outcomes were altered such as the child’s immune responses.

Although difference between early and late supplementation was only 30 days, infants of malnourished mothers showed some benefits from early supplementation. This supports the current practice of supplementing malnourished pregnant mothers. On reviewing the literature, food supplementation, particularly energy supplementation, has shown consistent benefits to birth weights though the increase was not always significant (Chapter 1.5). Apart from our study, only one study from a developing country, supplemented pregnant mothers with food and found a short term benefit to infants’ development (Joos et al 1983). Furthermore, no study reported any harmful effects from prenatal energy supplementation and famine studies suggest some long term deficits to mental health from hunger. Thus, if resources are available it is safe to start food supplementation (600 kcal) in early pregnancy in underweight mothers.

However the benefits of supplementation were limited, the effect size of early food was 0.2 and 0.1 of a standard deviation score for support test and cover test respectively.

I would strongly recommend continuing food supplementation to low BMI mothers and extending the present programme to include early pregnancy. It is also important to make it easier for the women to take the food as compliance was not good. One approach would be to allow the women to take home one week's supply at a time. However this would need piloting as sharing may be prevalent and reduce the impact. Changes in policy depend on availability of resources and the benefits have to be weighed against the costs. As the existing food supplementation program targeted malnourished mothers, only a slight change would be necessary to persuade mothers to start earlier. The food used in supplementation was of poor nutritional quality and needs improvement to include micronutrients and reduce monotony. Resources need to be committed to field trials to assess the effectiveness of using different ways of providing the food and different types of food.

In contrast to food supplementation, recommendations about prenatal multiple-micronutrient supplementation are less clear. Recently a combined report from two Nepalese studies raised questions about the safety of multiple-micronutrient supplementation in pregnant women (Christian et al, 2005). They found increased perinatal mortality in spite of increasing birth sizes and minimizing incidence of LBW.

Although some studies on prenatal multiple micronutrients have shown increases in birth weight, no effect on child development has so far been reported. The benefits we found were small and I would not recommend any change in policy at this stage until we follow these infants further and have data on all other consequences.

5.8. Future plans and research questions raised by this study

The developmental outcome due to early supplementation may change overtime. For example, Helland and colleagues (2001) supplemented mothers with fish oil from the second trimester of pregnancy until 3 months postpartum and failed to find any cognitive

benefit (novelty preference using Fagan test) to their infants at the age of 6 months. However at the age of 4 years, these children showed significantly higher intelligence scores (assessed by Kaufman Assessment Battery for Children) compared to the control group (Helland et al, 2003). In contrast Joos and colleagues (1983) found significantly higher motor scores (Bayley scale) in infants of food supplemented mothers around 8 months of age, but failed to find any benefit on intelligence score (Stanford Binet) at the age of 5 years. It is therefore necessary to follow these Bangladeshi children longitudinally to determine whether the small benefits found are not only sustainable but also whether the effect size changes.

So the questions arising from our findings are-

- (i) Will this beneficial effect increase with time or fade away?
- (ii) Is there any dose response?
- (iii) What is the predictive validity of the early developmental tests?
- (iv) Is supplementation protective against the effect of arsenic exposure on child development, which is highly prevalent in this region?

5.9. Conclusion from the study

In this study we found that early food and multiple-micronutrient supplementation during pregnancy was beneficial for the offspring's cognitive and motor development and behaviour ratings, but only when given to malnourished mothers. The effect size was very small and its clinical and public health importance is not clear. However, there was no placebo group and comparisons were made only with later food supplementation and iron and folate supplementation. In addition the compliance was only moderate and we lost smaller babies from the study so that the actual benefit would probably have been greater compared to placebo group.

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Annex

Annex 1

ICDDR,B: Centre for Health and Population Research

Combined Interventions to Promote Maternal and Infant Health: Effects Over a Pregnancy Cycle
and on Children 0-24 Months

WRITTEN CONSENT FORM

Thank you for participating in the study during pregnancy. We would like to invite you to continue to participate in the study even after child birth. I am sure you remember why we do this study, but let me repeat some of the information for your benefit. Good maternal nutrition and treatment of infection during pregnancy are very important for the health and well being of the mother and her baby. Poor maternal nutrition and infection during pregnancy are very common in Bangladesh, as in Matlab, which results in lack of energy/protein, vitamins and minerals. Because of this, a lot of illnesses and deaths take place among mothers and their babies.

ICDDR,B in collaboration with the Government of Bangladesh, UNICEF and collaborating universities in the United States and Europe is undertaking a study to improve maternal and infant nutritional status. You have participated in this during pregnancy, and it has focused the ongoing feeding program, tablets with vitamins and minerals, investigation regarding vaginal infection and advice regarding breast feeding. The “Pushti” program continues also after birth, you have received tablets with vitamins and minerals to take for some more months, and you will continue to receive either counselling to help you with breast-feeding of your baby or health education on care for yourself and the baby. We are now interested to study to what extent these interventions are benefiting your health and your child’s health and further development.

[*Only when delivering at sub-centre:* We will collect two tubes of blood from the cord (not from you or your child) and we will also take a small piece of the placenta in order to examine health conditions in the placenta. We will also take 3 ml of blood in a tube from your child’s veins at three different occasions during the first years in order to examine the effects of the food supplementation and the micronutrient tablets you received during pregnancy. We will examine the haemoglobin concentration on one of these occasions and inform you about the result. Other results will not be reported back, and we ask for your permission to use it for scientific purposes.]

[*Only when not delivering at sub-centre, during the 7-10 day home visit:* We will also take 3 ml of blood in a tube from your child’s veins at 6 months in order to examine the effects of the food supplementation and the micronutrient tablets you received during pregnancy. We will examine the haemoglobin concentration and inform you about the result. Other results will not be reported back, and we ask for your permission to use it for scientific purposes.]

You are familiar with ultrasound examination. We will examine your child with ultrasound on 3-4 occasions, and see how thymus, an organ that is involved in the defence against infections, is developing. This examination takes a few minutes and is not causing any harm for your child.

We will continue to measure weight and other body measurements on your child and also, for a few times, even your weight. We will also ask questions about your health and your child’s health, feeding and development. On three occasions during the first years we will observe how your child advances in movements and in play.

We will also collect 5.5 ml of blood (about a teaspoonful) from your veins. We will test this blood for anaemia, and tell the result. Other results will not be told, and we ask for your permission to use it for scientific purposes. We will also ask you to get a portion of

breast milk on three occasions, for analysis of effects of the food and micronutrient supplementation.

We assure you that we shall maintain the confidentiality about the information we collect from you. All records from this study at the Matlab Diarrhoea Hospital or the Dhaka offices of ICDDR,B will be kept private and in a locked location. Only people doing the study will be able to look at them. Any study records that are taken from ICDDR,B will not have any of the names of who took part in the study.

Your participation is absolutely voluntary. You are at liberty to withdraw from the study at any time during the study without any penalty or change in the routine care you or your child receives. If you decide not to take part in these parts of the study, it will not change the care you, your child or your family receives from ICDDR,B in any way. You will still receive our routine care and necessary support and treatment.

You may ask any questions regarding the study and I shall be happy to answer them for you. If you have any problems or questions you can contact your home health care worker, or contact Matlab Hospital of ICDDR,B or Dr. Lars Ake Persson at the following phone number at any time: 988 5155 (Dhaka).

- | | | |
|--|-----|----|
| • Do you have any questions? | Yes | No |
| • Do you agree to participate in this study? | Yes | No |

Signature of the witness
woman

(Paramedic) Date: _____

Signature/thumb impression of pregnant

Annex 2

International Centre for Diarrhoeal Disease Research, Bangladesh
Combined Interventions to Promote Maternal and Infant Health – a study in Matlab Study

Child Development Form (Problem Solving Test)

Child Name _____

ID:

a) Follow up visit number

b) Scheduled Visit 1 = Yes , 2 = Revisit

Support: Problem Solving Test	a) Trl-1	b) Trl-2	c) Trl-3	d) Trl-4	e) Trl-5
PS01. Support-Cloth					
PS02. Support-Fixation					
PS03. Support-Toy					
PS04. Support Total Score					
PS05. Support Intentional Solution (0=no, 1=partial, 2=Full)					
PS06. Support Cloth-Toy Cont. Time					
PS07. Support Reach (1=yes, 2=no)					
PS08. Support Fall off (1=yes, 2=no)					

Cover: Problem Solving Test	a) Trl-1	b) Trl-2	c) Trl-3	d) Trl-4	e) Trl-5
PS09. Cover-Cloth					
PS10. Cover -Fixation					
PS11. Cover -Toy					
PS12. Cover Total Score					
PS13. Cover Intentional Solution (0=no, 1=partial, 2=Full)					
PS14. Cover Cloth-Toy Cont. Time					

PS15. Problem Solving Behavior	a) Approach	b) Activity	c) Vocalization	d) Emotion

PS16. Bayley Motor Raw Score

PS17. Bayley Motor Index Score

PS18. Bayley Motor Behavior	a) Activity	b) Vocalization	c) Emotion	d) Cooperation

PS19. Comments (If any):

PS20. Developmental age

Annex 3

International Centre for Diarrhoeal Disease Research, Bangladesh
Combined Interventions to Promote Maternal and Infant Health – a study in Matlab Study

Child Development Form (Milestones)

Child Name _____

ID:

a) Follow up visit number

b) Scheduled Visit 1 = Yes , 2 = Revisit

TEST ITEM	EXAMINER REPORT a) Observed 1=no (inability), 2= yes 3= refusal, 4= very sick 9= unable to test (any other reason: absence, mother's refusal, sleeping, etc.)	CARETEKER REPORT	
		(b) Precise Date of first achievement (Mother's Recall)	(c) Type of Record 1= tested & recorded 2= recalled 3= only tested
1. Holds head erect (15 sec)	<input type="text"/>	<input type="text"/>	<input type="text"/>
2. Lifts Head & Chest	<input type="text"/>	<input type="text"/>	<input type="text"/>
3. Sits with hand support	<input type="text"/>	<input type="text"/>	<input type="text"/>
4. Picks up cube	<input type="text"/>	<input type="text"/>	<input type="text"/>
5. Transfers object	<input type="text"/>	<input type="text"/>	<input type="text"/>
6. Sits without support	<input type="text"/>	<input type="text"/>	<input type="text"/>
7a.Handknee crawl (3 rws)	<input type="text"/>	<input type="text"/>	<input type="text"/>
7b. Other crawl (3 rws)	<input type="text"/>	<input type="text"/>	<input type="text"/>
8. Pulls self to stand	<input type="text"/>	<input type="text"/>	<input type="text"/>
9. Stands assisted	<input type="text"/>	<input type="text"/>	<input type="text"/>
10. Walks assisted	<input type="text"/>	<input type="text"/>	<input type="text"/>
11. Stands alone	<input type="text"/>	<input type="text"/>	<input type="text"/>
12. Walks alone	<input type="text"/>	<input type="text"/>	<input type="text"/>
<div style="display: flex; justify-content: space-between;"> <div style="width: 24%;"> 13. Child's State (a) First Scale 1= Drowsy 2= Awake <input type="text"/> </div> <div style="width: 24%;"> (b) Second Scale 1= Calm 2= Fussy 3= Crying <input type="text"/> </div> <div style="width: 24%;"> (c) Fussiness 1= Most of the time 2= Some of the time 3= Not at all <input type="text"/> </div> <div style="width: 24%;"> (d) Happiness 1= No sign 2= a little time & alert 3= Most time <input type="text"/> </div> </div>			
14. REMARK			

Annex 4

Problem Solving Test Instructions

MINIMat Child Development Component
ICDDR,B, International Centre for Health and Population Research

Means end Problem Solving Test (Scoring &Instructions)

Designed by Willatts P
Dandee University, UK

Cloth Behavior:

Start Time-

Start counting trial-time immediately after offering the tray towards the child.

If the child touches the cloth within 30 sec after offering the tray, start counting cloth-time immediately after touching the cloth.

End Time-

- 30 sec after start.
- If toy within reach/ touch within 30 sec after trial-start.
- If the toy falls off the table.

Scoring-

Score 0

- No contact with cloth
- Toy not brought within reach
- Infant clearly plays or examines the cloth at anytime before the toy within reach ie mouthing the cloth.

Score 1

- Infant pulls the cloth without any play or examination, and brings the toy within reach however before this begins an activity that might be play or examining, but does not carry it through to completion (within 30 sec)
- Lets go off the cloth for more than 1 sec before the toy comes within reach

Score 2

- Infant pulls the cloth without any play or examination, and brings the toy within reach.
- Short breaks in contact of 1 sec a less are allowed provided the infant immediately regains contact.
- Pauses of any duration between movements of cloth are permitted.
- Toy pulled off table on side where child is sitting.

Fixation

Start Time –

- Start counting fixation-time immediately after offering the tray towards the child.
- If the child touches the cloth within 30 sec after offering the tray, restart counting fixation-time immediately after touching the cloth.

End Time-

- 30 sec after start.
- Toy touch within 30 sec after trial-start.
- Toy off the table.

Score –***Score 0***

- If infant looks away from the toy for more than two seconds

Score 1

- If infant briefly looks away from the toy, but looks back within two seconds.

Score 2

- If infant fixates the toy continuously until touched or falls off table.

Toy Behavior

Score if the toy is picked up or trial ends (30 sec after trial starts).

Score –***Score 0***

- Infant fails to contact the toy.
- Only touches the toy and makes no attempt at grasping.
- The cloth is pulled too far and toy is dragged off the edge of table.

Score 1

- Infant attempts to grasp the toy, but does not pick it up.

Score 2

- Infant grasps the toy and pick it up

Test Administration

Aims - The test will be observed for two things -

- For behavior and
- For problem solving.

Preliminary preparation –

- Write down the ID number, date of birth and date of examination on the nameplate of the child and do the video recording.
- Check the video camera before each test for electric connections, battery charges, time setting, positioning and whether the tape is in the position after the previous recording.
- Then call the mother and seat her in the chair. Readjust the camera so that the child's face and the tray are clearly visible in the TV screen for recording.
- Press the Recording button and start testing.
- Stop recording after the end of testing.

In first 5 minutes –

- Seat the child comfortably on mothers lap so that his chest touches the edge of the table.
- Describe the mother the purpose of the test.
- Give necessary instruction to the mother including not to help the child, not to guide the child during play and hold the child in such a way so that his hands are free to play.
- Ask whether it is the time for his sleeping/ feeding /toileting.
- Interact twice with the child and make eye contact.
- Start pretrial.

Practice sessions –

- Two practice sessions with cloth.
 - Purpose is to make familiarize the child with cloth
 - Give 20 seconds for each practice session.
 - If the child does not touch it, hand him the cloth directly.
 - Give it directly on the table, not on the tray.
- Two practice sessions with toy.
 - Purpose is to make familiarize the child with toy, is to get the most interesting toy for him and is to find the distance of within-reach.
 - Give 20 seconds play for each trial, start time counting soon after he manipulates the toy. If the child is very keen on it give some extra time to satisfy his desire.
 - Do not use/ show other toys to get the toy from him.
 - If the child does not touch it, hand him the toy directly.
 - Each time give the toy on the tray to make him familiarize with the test procedure.

Trials –

- Give 4 trials.
- Do not talk, make sounds or make eye contact with the child during trials.
- Make less possible interaction with the child in between trials.
- Hand the toy to the child after each trial and let him play for 20 seconds.
- If he loses interest on that toy, change the toy and allow him to play with new toy for 20 seconds.
- If the child cries or fusses, try to continue the test if possible but do not change the tests (from support to search). Try to console him with other toys. If fussing throughout the test, stop testing and ask her to bring the baby again if possible in the same day or on the next day.

After Test –

- Score on videos on the same day.
- Transfer the recorded tests from camera tape to VHS tapes prior to scoring.

Annex 5

Behavior Ratings

MINIMat Child Development Component
ICDDR,B, International Centre for Health and Population Research

Behavior Ratings

Approach: Initial response to the examiner. The examiner addresses a few introductions, remarks to the child and then talks with the mother after giving the child a toy.

Response in the first 5 to 10 minutes is rated. It should be rated immediately, not at the end of the test.

1. Avoiding: shows stray signs of fear- clinging onto the mother/ fussing/ looking away, withdrawing.
2. Between 1 and 3.
3. Hesitant: Some fear/obviously worried/wary and watchful/not happy/not smiling/not fussing/not readily playing but may be slight touching of toy. May look fleetingly at examiner.
4. Between 3 and 5.
5. Accepting: No sign of fear but aware of examiner/not offering/not vocalizing or smiling at examiner/but looking at her from time to time without fear. Plays with toy but not with vigour.
6. Between 5 and 7.
7. Friendly: Not afraid. May smile or vocalize or offer toy to examiner after a few minutes, plays with toy readily.
8. Between 7 and 9.
9. Inviting: Fully accepts examiner, happily. Interacts with her smiling, vocalizing and/or approaching. Obviously enjoys toy, May show enthusiasm in playing

General emotional tone: This scale refers to how unhappy and fussy or cheerful and happy the infant appeared during the examination.

1. Child seems unhappy throughout the assessment, gets very upset, cries and fusses for long periods or frequently may protest and wail.
2. Between 1 and 3.
3. At times rather unhappy begins to fuss often with cries, short verbal protests but may respond happily to some procedures.
4. Between 3 and 5.
5. Moderately happy or contented (smiles and positively vocalizes in response to some tasks), may become upset occasionally but recovers fairly easily.
6. Between 5 and 7.
7. Generally appears to be in a happy state of well beginning, smiles often with some excitement only becomes briefly unhappy once or twice during the whole assessment.
8. Between 7 and 9.
9. Radiates happiness, highly excited, nothing is upsetting (never becomes upset), animated, expressive, smiling, and gleeful.

Activity: This scale refers to how physically active the infant was during the testing (gross motor activity).

1. Very still, little gross motor movement stays quietly in one place, with practically no self initiated movement, never wiggles around.
2. Between 1 and 3.
3. Usually quiet and inactive, rarely wiggles but responds appropriately in situations calling for some gross motor activities (motor tasks)
4. Between 3 and 5.
5. Moderate activity, wiggles occasionally and may get up or change position a number of times, can be quieted for sedentary tests without much difficulty.
6. Between 5 and 7.
7. In action during much of the assessment period, gets up frequently, moves around the room, wiggles, movements are consolable and can be quieted for sedentary tests, however with difficulties sometimes.
8. Between 7 and 9.
9. Overactive, on the move all the time, wiggles a lot, cannot be quieted for most of the sedentary tests.

For assessing activities during problem solving test

1. Very still, very little movement of trunk and legs, stays quietly in one place, with little self initiated movement except necessary to do the task
2. Between 1 and 3.
3. Moderate activity, wiggles occasionally and may get up or change position a few times.
4. Between 3 and 5.
5. Active, most of the time, wiggles a lot, change position often may stand up on lap or try to get on table.

Cooperativeness: This is a measure of how well the infant cooperates with the examiner and complies with his/her requests.

1. Resists all suggestions or requests, which are assessment related, very resisting and uncooperative.
2. Between 1 and 3.
3. Refuses or resists several specific examinations initially or refuses to cooperate during part of the session (e.g. initially or towards the end)
4. Between 3 and 5.
5. Accepts the assessment or situation, neither cooperative nor resistant in relation to examiner, may occasionally say 'No' but will conform.
6. Between 5 and 7.
7. Seems to enjoy the interaction with the examiner, is happy to participate most of the time.
8. Between 7 and 9.
9. Enjoys the session and always complies, readily accepts the examiner's manipulations

Vocalising: Vocalisations refer to non-crying utterances or to recognisable utterances embedded in crying. These may be cooing, babbling, consonant sounds or words. Crying, per se, no matter how varied, does not qualify

1. Definitely quiet.
2. Between 1 and 3.
3. Few vocalisations and have short durations
4. Between 3 and 5.
5. Vocalisation occur as a part of activities but too intermittent to constitute vocal excitements, chatter or the like
6. Between 5 and 7.
7. Vocalisations constitute an obvious part of the infant's activity: infant vocalises for the sake of vocalisation.
8. Between 7 and 9.
9. Excessive vocalizations, high vocal excitement.

Annex 6

**Milestone Training Manual for MINIMat
Field Workers**

MINIMat Child Development Component
ICDDR,B, International Centre for Health and Population Research

Training Manual For Milestone

What is Milestone?

The sequence through which babies gain voluntary control over their own movement is referred to as motor development. Motor behavior such as lifting head, sitting, crawling, walking etc. is one aspect of child's development and known as 'motor milestone of development'.

Children achieve maturation of different functions at predictable age within a range of a few months. During the first two years of life parents count gross motor milestone as an indicator of their children's normal progress, which is sequential in manner with narrow range of individual variation in normal children.

Purpose of measuring the Milestone:

Brainstem mediated primitive reflexes undergo significant evolution during the first year of life. Attainment of gross motor skills indicates not only maturation of the complex neurological development, but also has great impact on children's cognitive development and emotion. The aim of the present study is

- To see the effect of different interventions during pregnancy on their children's attainment of milestones.
- To correlate the motor milestone with future cognitive development

Milestones, selected for assessment:

We have selected 12 milestones including six universal gross motor milestones (asterisked) used in WHO Multicentre Growth Reference Study (MGRS):

1. Holds head erect and steady (15 sec)
2. Lifts head and upper trunk on tummy/ stomach
3. Sits with support
4. Picks up toy /cube
5. Transfers object from hand to hand
6. Sits without support*
- 7a. Moves forward or backward easily by hand and knee crawl, with tummy/stomach not touching the ground (at least 3 in a row)*
- 7b. Moves forward or backward easily by any means other than hand and knee crawl (using stomach & arms, buttock & hand/leg, hands & feet etc- at least 3 in a row)
8. Pulls self to stand
9. Stands with assistance*
10. Walks with assistance*
11. Stands alone*
12. Walks alone*

NB: Among these 'Picking cube' and 'Transfer object' are combinations of fine motor and mental activities but the rest are gross motor activities.

Site:

The study will be conducted at Matlab, a rural area of Bangladesh, where ICDDR, B's principal field station for conducting community-based research on health and population has been functioning since last 20 years. The Matlab, an Upo-Zilla in Chandpur district lies in the east-central plain of Bangladesh and forms a delta by two major rivers, the Meghna and the Padma. It is situated 53 kilometers southeast of Dhaka; the capital of Bangladesh and it comprises a total of 142 villages and covers an area of 184 square kilometers.

Population:

The density of population of the study area is about 1100 per square kilometre. In the study area, the people live in clusters of patrilineally related households called *bari*. The literal meaning of the *bari* is homestead. Each *bari* is formed with an average of six households which holds about thirty people. These households usually own one- or two-room houses, mostly with mud floor, interlaced bamboo walls and thatched grass or galvanized iron (tin) roof. The main economic activities of the area are farming, fishing, trading, and crafts. The study sample will include 3000 mother-infant pairs.

Materials need to be carried with Health Workers (HW):

1. Toy- 2 cubes, 1 plastic ring, 2 squeeze toys and 1 rattle.
2. A plastic play-mat
3. A one paged structured questionnaire
4. A plastic covered pictorial calendar, which will be given to mothers at the very 1st visit.
5. One laminated Milestone instruction sheet with all 12 milestones.

General Instructions for HW:

1. Approach to the mother/respondent
 - Introduce yourself as a staff of ICDDR
 - Describe briefly the purpose of your visit to the mother
 - Always practice courtesy, politeness and respect.
 - Keep in mind that you are a visitor in their home and you are asking them to spare their valuable time. So, deal with them in a very friendly and cordial manner
 - Respect privacy. Never enter the house without the mother's permission- even if you visited them before.
 - Never argue with the respondent even if some of the answers and behavior you may not like.
 - Always talk to the mother with smiling face, in front of the child before approaching the child
 - Ask her about the health and well being of the child
 - Request for her optimum co-operation to perform the tests after describing her what she has to do. Always thank mother for her time and co-operation.

2. Approach to the child

Ideally, the child should be awake and alert & calm prior to testing. Drowsiness, fussiness, crying and minor illnesses are not reasons for not testing if they do not interfere testing procedure. However, if they interfere, the child should be retested when s/he is calm or recovered. Test the infants if they are awake, happy and playful.

- Ensure that the child is not hungry and not too sick to perform the test.
- If the child is suffering from minor illnesses (Cough, cold, runny nose, mild diarrhoea etc), which do not interfere with child's activity, continue the test.
- Always involve mothers or caretaker during performing the tests.
- If the child is asleep, then the child should not be tested. In that case do the anthropometric measurements first and then test him if s/he is awakened and settled, otherwise revisit the child when he is awake.

3. Completion of Forms:

- Milestones should be assessed once in a month along with monthly antropometric measurement.
- Fill out a one-paged form for this purpose.
- The form includes both direct observation and mothers' report (recall)
- In addition also tick the achieved milestone in the pictorial calendar given to the mother during the 1st visit so that you can keep a track of the achieved milestone and avoid repetition in the next visit.
- Maintain neatness and cleanliness in your work
- Never rub or overwrite a figure. Put a single line through the error and write the correct figure above it. Initial the correction.
- Check each form for missing values, abnormal values and translocation errors.

4. Maintenance of Data Forms (Data Hygiene):

- At the end of each day check each form for four things:
 - a) Missing information eg. Name, date etc
 - b) Wrong/Abnormal information eg. Age, ID number etc.
 - c) Translocation errors. These are errors where information is shifted to another row and carried on for the remainder of the form.
- Always write in a clear legible manner. If you make a mistake, correct it. and initial the correction so that we know who made the correction (the HW or SFRA)
- Report any error to SFRA. If you are unsure of the correct data, do not try to guess. Make arrangement for retesting.
- After your checkup, submit the filled out forms to the respective SFRA for re-check before sending for computer entry.

Description of testing procedure:

The motor milestones generally follow a sequential pattern. However sometimes sequence between two or more milestone may be reversed or skipped. So HWs should not be worried about that. It is preferable to request the mother or caretaker to elicit the milestone. Particularly this is important in case of older infants who develop stranger's anxiety usually after 9 months.

In case of sick, fussy, sleepy or hungry child sometimes it will be difficult to test the desired milestone. In that case if possible take sufficient time to calm the child before performing the test. If these interfere with milestone test, HWs should try to revisit the child when s/he is settled.

See below the test description

1. Holds head erect and steady for 15 seconds:

Description: At this stage infant tries to balance his head to keep it erect.

Administration: Pick up the child by placing a your hands under her arms and around her body. Extend your fingers upwards along the back of the child's neck to support her head. Hold the child in an upright position with her head resting on your shoulder, moving one hand down to support the child's back and moving the other hand up to support her neck.

Carefully remove your hand from the child's neck according her ability to lift her head and keep it erect.

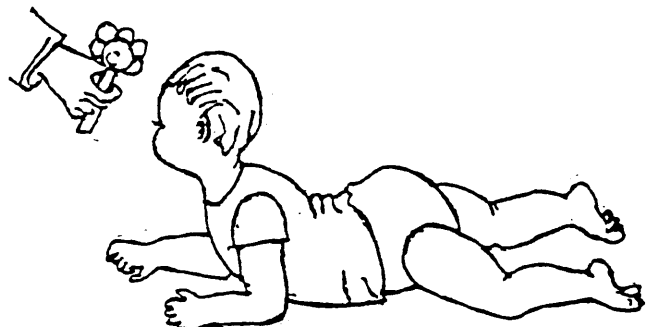


Scoring: Give credit if the child holds her erect and steady for approximately 15 seconds after you remove your hand from her neck.

2. Lifts head and upper trunk on stomach:

Description: At this stage an infant can lift his/her head, shoulder and chest off the exam surface in a prone position.

Administration: Ask the mother to place the child lying on his tummy/stomach and observe if he can lift his head and upper trunk from the exam surface, by pushing himself up on his elbows or forearms. If he doesn't raise his head, draw his attention by dangling a rattle above his head.



Score if:

- a) Head and part of the upper trunk are above the level of forearms, legs and tummy.
- b) The angle of the trunk with the floor should be at least 45°.

3. Sits with support:

Description: At this stage the child is able to sit by balancing the weight of the trunk and head with some support by placing his arms or hands on the ground or on his body. One of the lower limbs is usually flexed. S/he sits up straight or with the head may lean forward.

Administration: Sit the child on the bed/mat so that his legs are slightly bent at an angle of 45°; provide support by placing your hand around the child's lower back and give a support so that the child can sit. Then gradually and slowly take off your support and see whether she can adjust to sitting posture by supporting himself with hand on the ground or placing hands on any part of his body. Then begin timing.



Score if:

- a) The child sits alone for at least 10 sec.
- b) The child may use his hands to support his weight. The child may sit with a rounded back or wobble

4. Picks up toy /cube

Description: At this stage infants can grasp objects and held in the center of the palm by using some or all fingers.

Administration: Let the child sit comfortably on the bed so that she does not engage her/his hands in balancing. Alternatively, seat her on mother's lap if she cannot sit on her own. Bang 2 cubes together and draw her attention and then place them in front of her/or on the tester's palm. Make sure that the child sees the cube. If the child does not pick up the cube, attract her attention again moving the cubes and making a noise.



Score if:

- a) The child picks up the cube using one or both hands.
- b) Do not credit if the child presses the toy against her trunk in order to pick it up or hold it.

5. Sits without support:

Description: At this stage the child is able to balance the weight of the trunk and head without any external support or the use of arms or hands. S/he sits up straight with the head erect (and thus, is not leaning forwards). One of the lower limbs is usually flexed.

Administration: Facing the child and smiling place the child in a sitting position. Then give the child a toy to handle with both hands so that s/he is not able to use arms to support her/himself up.



Score if:

- a) Child's head is erect.
- b) Child does not use arms or hands to balance body or support position.
- c) Child sits up straight for at least 10 seconds.

6. Transfers object from hand to hand:

Description: At this stage the infant is able to bring her/his two hands together in midline and feel and manipulate. If either hand is holding together it may pass it to the other hand and so begins the process of transfer from hand to hand.

Administration: Let the infant sit comfortably on the bed or seat her on mother's lap. Give a cube/toy to the child and while she is holding it, observe whether she transfers the toy from one hand to another. Usually, it takes sometime to see this happening, so watch the child for at least 5-10 minutes.



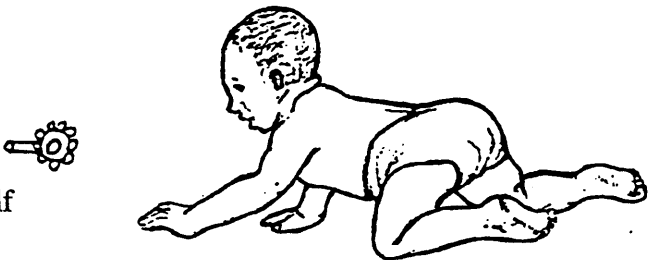
Score if:

- a) The child transfers the toy from one hand to another.
- b) Do not credit if the transfer occurs only when the child's free hand comes into contact with the toy by chance.
- c) Do not credit if the child releases the toy with one hand, then picks it up with the other hand
- d) Do not credit if the child uses another part of his body (e.g., mouth or trunk) to facilitate the transfer.

7a. Moves forward or backward easily by hand and knee crawl, with abdomen not touching the ground at least 3 in a row

Description: This is a phase of more organised prone movement that refers to the palm-knee position, with alternating movements of upper and lower limbs: the right arm and left leg move forward or backward synchronously and vice versa in similarly ordered consecutive movements. In some exceptional cases, the child may show this movement by using other combination like buttocks-legs or hands-feet.

Administration: Place the child in prone position with abdomen above the supporting surface and place yourself in front of the child (about 4 to 5 ft away). If the child does not crawl spontaneously, show her a toy or object that attracts her visual attention and keep the toy just outside her reach, so that he attempts to hold it. Sometimes with the help of the caretaker, try to coax the child to crawl towards the toy and grab it.



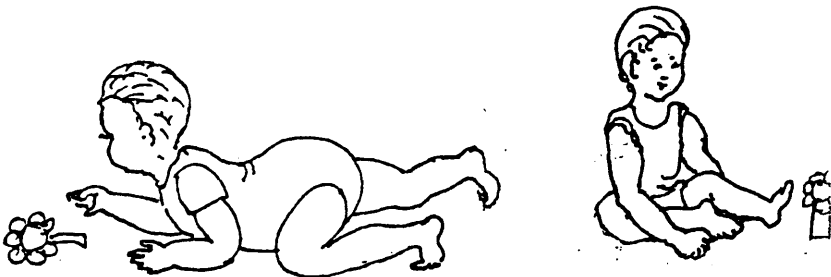
Score if:

- a) At least three alternating movement forward or backward in a row on hands and knees or in the way described above.
- b) Child's abdomen does not touch the supporting surface.
- c) Continuous and consecutive movements at least three in a row.

7b. Moves forward or backward easily by any means other than hand and knee crawl (using stomach & arms, buttock & hand/leg, hands & feet etc- at least 3 in a row)

Description: This is a phase of more organised prone movement that refers to the palm-knee position, with alternating movements of upper and lower limbs: the right arm and left leg move forward or backward synchronously and vice versa in similarly ordered consecutive movements. In some exceptional cases, the child may show this movement by using other combination like buttocks-legs or hands-feet.

Administration: Place the child in prone position with abdomen above the supporting surface and place yourself in front of the child (about 4 to 5 ft away). If the child does not crawl spontaneously, show her a toy or object that attracts her visual attention and keep the toy just outside her reach, so that he attempts to hold it. Sometimes with the help of the caretaker, try to coax the child to crawl towards the toy and grab it.



Score if:

- d) At least three alternating movement forward or backward in a row on hands and knees or in the way described above.
- e) Child's abdomen does not touch the supporting surface.
- f) Continuous and consecutive movements at least ten in a row.

8. Pulls self to stand:

Description: At this stage babies acquire an ability to pull themselves up from sitting to standing position.

Administration: Place the child near to any household furniture (chair, bench and tool), which the child can hold in an attempt to pull her/him up. Observe if she spontaneously pulls her/himself to a standing position using any convenient household objects for support.



If the child does not spontaneously raise her/himself to standing position, place the child on the floor and shake a toy above and in front of her/him. Keep the toy on the edge of the chair or bench and see whether he tries to hold that by pulling herself to a standing position.

Score if:

- a) The child could raise her/himself to a standing position by holding a chair or any other convenient objects for support.
- b) The child should stand for few seconds after pulling herself to standing position.

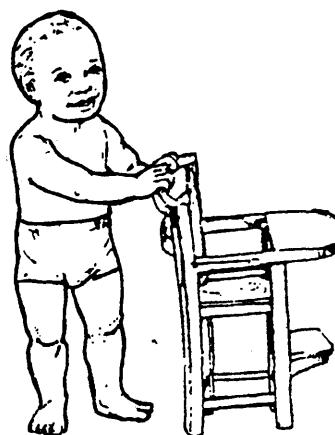
9. Stands with assistance:

Description: This is the first direct step toward erect bipedal locomotion where the child is for the first time challenged to maintain some balance of her/his whole body weight so that s/he can move forward. At issue is whether the child can actually support her/his weight if s/he is holding on to a stable object (eg furniture) with both hands without leaning over or resting her/his body on the stable object.

Administration

Place the child in a standing position so that his legs support of her/his body weight. The child is placed at a distance from which both hands, but not the body, can reach and hold onto a stable object (e.g. furniture).

Thus most of the body weight is supported by the child's own feet. The fieldworker should check that the child is not leaning over or resting her/his body on the stable object. The height of the stable object should be about the level with the child's stomach.



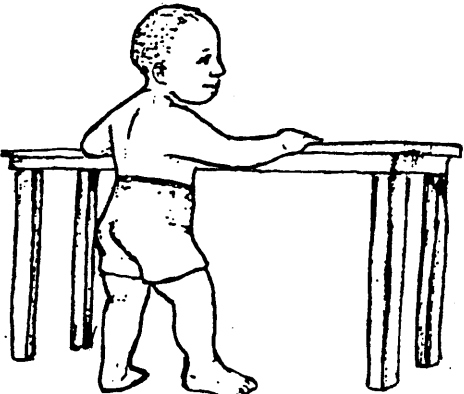
Score if:

- a) Child is in upright position on both feet.
- b) Child holds onto a stable object with both hands without leaning on it.
- c) Child's body does not touch the stable object.
- d) Child's legs support most of the child's body weight.
- e) Child thus stands with assistance for at least 10 seconds.

10. Walks with assistance:

Description: There is a deliberate attempt to make stepping movements and to make postural adjustments towards this end. The child is making sideway or forwards steps by holding onto a stable object (eg furniture) for support with one or both hands.

Administration: Place the child in a standing position so that the legs support most of her/his body weight. The child is placed at a distance from which s/he can reach and hold onto a stable object (e.g. furniture) with one or both hands. If the child does not move spontaneously, show the child a toy or object that attracts the child's visual attention. Then (sometimes with the help of the caretaker) try to coax the child to walk towards the toy and grab it. The height of the stable object should be about the level with the child's stomach.



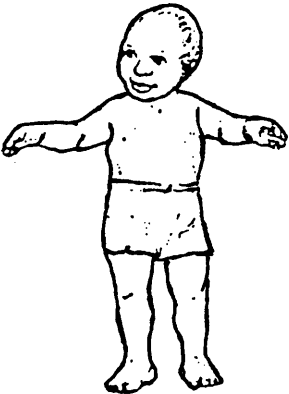
Score if:

- a) Child is in upright position with her/his back straight.
- b) Child makes sideways or forward steps by holding onto a stable object with one or both hands.
- c) One leg moves forward while the other supports part of the body weight.
- d) Child takes at least five steps in this manner.

11. Stands alone:

Description: The child shows the capacity of both equilibration and sustaining the bodyweight over the feet. In this position the child show no flexion, the child is standing on her/his feet (not on the toes) without leaning over or holding onto an object. The child maintains continuous balance independently.

Administration: Place the child with both her/his feet flat on the floor and support her/him to an erect position by holding lightly with her/his both hands. Then withdraw the support (your hand) gradually and temporarily to determine whether she can modify posture, adjust to his position and stand-alone for at least 10 seconds.



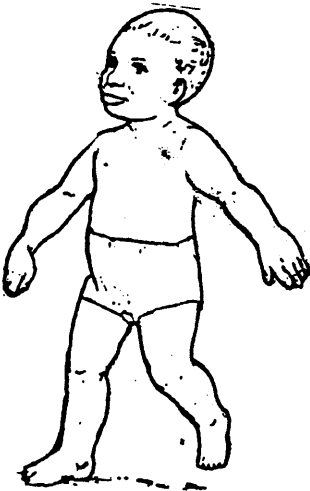
Score if:

- a) Child is in upright position on both feet (not on the toes) with her/his back straight.
- b) Child's legs support 100% of the child's weight.
- c) There is no contact with person or object.
- d) Child stands alone for at least 10 seconds.

12. Walks alone:

Description: The child shows the capacity to balance his /her and to control her/his forward stepping movements. There is no need of assistance because both the postural adjustment and the stepping movement engage in independent walking. An important indicator in this phase of erect locomotion is that movements of the entire body do not accompany the child's stepping movements. (NB This phase does not refer to the child's first independent steps when s/he is able to take 3-4 uncertain steps towards the adult's outstretched hands.)

Administration: Place the child in an erect position out of reach any supporting object. Then place yourself in front of the child (4 to 5 ft. away) and call the child to come towards you. Sometimes the caretaker needs to encourage the child.



Score if:

- a) Child is in upright position with her/his back straight.
- b) One leg moves forward while the other supports most of the body weight.
- c) There is no contact with person or object.
- d) Child takes at least five steps independently.

Form that need to be filled out:

The form includes the following parts

- a) *Particulars of study subject:*
In data set- write Study ID number, Follow up visit number and whether it is a scheduled or revisit accordingly
- b) *Test items-* 12 milestones in sequence
- c) *Observation part-* Interviewers' observation at the day of visit
- d) *Mothers' statement-* whether the particular milestone achieved.
- e) *Mothers' recall-* probable date of attainment of the particular milestone
- f) *Childs emotional state during test* (Rate the child's emotional state during testing of milestones according to the following two and three three-point scales)
- g) *Any comment regarding test-* whether the test was done, probable reasons of not testing.

Annex 7

Milestone Calendar for Mothers

MINIMat Child Development Component
ICDDR,B, International Centre for Health and Population Research

Milestone Calendar ১০৬-২৩.১০.০২
MINIMat Child development Component

STUDY ID # 20222

১. ১৫. সে: মাথা সোজা রাখা

① Holds head erect 15 sec

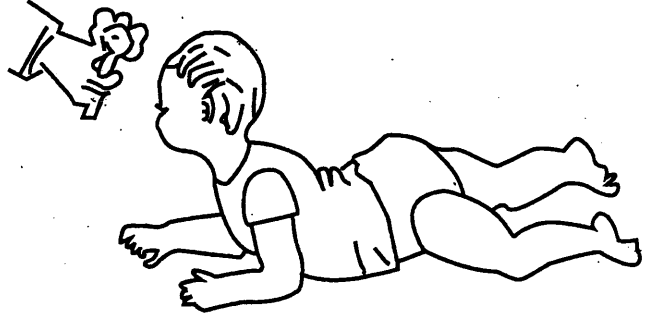
১ম ডিজিট: 25.01.03 ☒
২য় ডিজিট: 16.02.03 ☒
৩য় ডিজিট: ☐



২. মাথা ও বুক উচু করা

② Lifts head and upper chest

১ম ডিজিট: 25.01.03 ☒
২য় ডিজিট: 16.02.03 ☒
৩য় ডিজিট: ☐



৩. কোনকিছুর সাহায্যে বসা

③ Sits with support

১ম ডিজিট: 25.01.03 ☒
২য় ডিজিট: 16.02.03 ☒
৩য় ডিজিট: 20.03.03 ☒
৪ম ডিজিট: 24.04.03 ☒



৪. খেলনা বা গুটি তোলা

④ Picks cube

১ম ডিজিট: 25.01.03 ☒
২য় ডিজিট: 16.02.03 ☒
৩য় ডিজিট: 20.03.03 ☒
৪ম ডিজিট: 24.04.03 ☒
৫ম ডিজিট: 22.05.03 ☒



৫. এক হাত থেকে অন্যহাতে খেলনা নেয়া

⑤ Transfers from hand to hand

১ম ডিজিট: 16.02.03 ☒
২য় ডিজিট: 20.03.03 ☒
৩য় ডিজিট: 24.04.03 ☒
৪ম ডিজিট: 22.05.03 ☒
৫ম ডিজিট: 10.06.03 ☒



৬. কোনকিছুর সাহায্য ছাড়া একা বসতে পারা

⑥ Sits alone

১ম ডিজিট: 16.02.03 ☒
২য় ডিজিট: 20.03.03 ☒
৩য় ডিজিট: 24.04.03 ☒
৪ম ডিজিট: 22.05.03 ☒
৫ম ডিজিট: 10.06.03 ☒
৬ম ডিজিট: 20.07.03 ☒

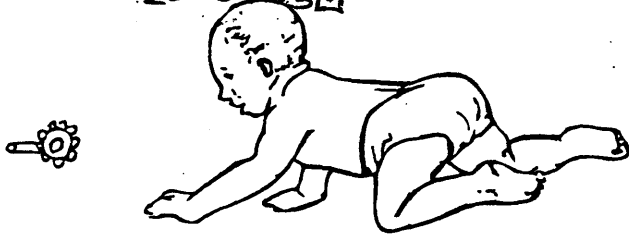


৭ (এ) হাত ও হাঁটুর সাহায্যে হামাগুড়ি (৩ ধাপ)

১ম ভিজিট: 20.03.04 ২য় ভিজিট: 21.04.04 ৩য় ভিজিট: 22.05.04

৪ম ভিজিট: 22.05.04 ৫ম ভিজিট: 21.06.04 ৬ম ভিজিট: 20.07.04

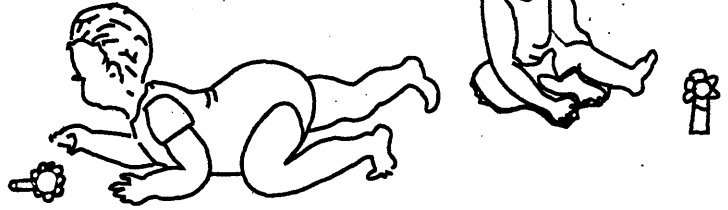
৭ম ভিজিট: 20.07.04 ৮ম ভিজিট: 20.07.04 ৯ম ভিজিট: 20.07.04



৭ (বি) অন্যান্য ধরনের হামাগুড়ি (৩ ধাপ)

১ম ভিজিট: 20.03.03 ২য় ভিজিট: 21.04.03 ৩য় ভিজিট: 22.05.03

৪ম ভিজিট: 22.05.03 ৫ম ভিজিট: 21.06.03 ৬ম ভিজিট: 20.07.03



৮. কোন কিছু ধরে বসা থেকে উঠা

৭ (এ) Pulls Self to Stand

১ম ভিজিট: 22.05.03 ২য় ভিজিট: 21.06.03 ৩য় ভিজিট: 20.07.03

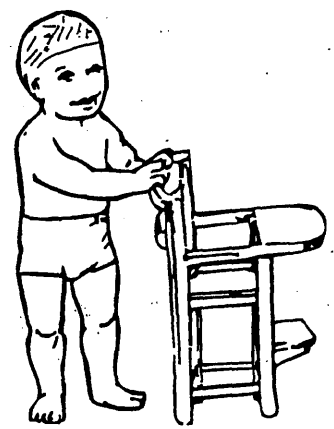


৯. কোন কিছু ধরে দাঁড়ানো

৭ (বি) Stands with Support

১ম ভিজিট: 22.05.03 ২য় ভিজিট: 21.06.03 ৩য় ভিজিট: 20.07.03

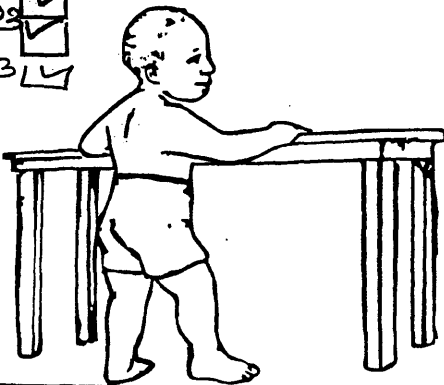
৪ম ভিজিট: 21.08.03 ৫ম ভিজিট: 17.09.03



১০. কোন কিছু ধরে হাটা

৭ (এ) Walks With Support

১ম ভিজিট: 20.07.03 ২য় ভিজিট: 21.08.03 ৩য় ভিজিট: 17.09.03



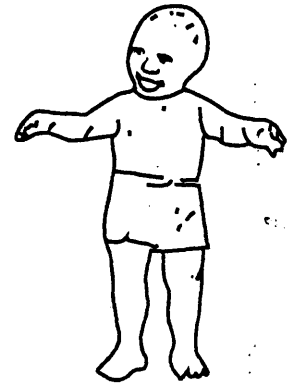
১১. কোন কিছুর সাহায্য ছাড়া একা একা দাঁড়ানো

৭ (বি) Stands alone

১ম ভিজিট: 20.07.03 ২য় ভিজিট: 21.08.03 ৩য় ভিজিট: 17.09.03

৪ম ভিজিট: 23.10.03 ৫ম ভিজিট: 15.01.04

= 01.10.03



১২. কোন কিছুর সাহায্য ছাড়া একা একা হাটা

৭ (এ) Walks alone

১ম ভিজিট: 20.07.03 ২য় ভিজিট: 21.08.03 ৩য় ভিজিট: 17.09.03

৪ম ভিজিট: 23.10.03 ৫ম ভিজিট: 15.01.04

= 27.11.03

